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PULSED ELECTRIC FIELD (P.E.F) AND PECTINASE FOR THE EXTRACTION OF POLYPHENOLS FROM GRAPE POMACE AND PEEL

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by

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PULSED ELECTRIC FIELD (P.E.F) AND PECTINASE FOR THE EXTRACTION OF POLYPHENOLS FROM GRAPE POMACE AND PEEL

Julien Khalil, M.S.

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Adviser: Durward A. Smith

Grapes are an important source of bioactive compounds such as gallic acid, resveratrol, and catechin. The consumption of grapes is associated with a lower risk of diseases such as cardiovascular diseases and some types of cancer. Grape pomace and skins are good sources of many phytochemicals known for their antioxidant potential.

In this research, the peel of the Chilean “Flame” grape cultivar and the pomace of St. Croix, Frontenac and St. Pepin grape cultivars were subjected to a pulsed electric field (5 KV, 1 μFarad, 20 Pulses) and to an enzymatic treatment (Pectinase, 5KU). The total phenolic content, determined in gallic acid equivalents using the Folin-Ciocalteau assay was analyzed. In addition to that, some of the individual phenolics present in the extracts were identified and quantified using HPLC. Finally, the antioxidant potential of the extracts was calculated using the FRAP assay.

This research explored the possibility of establishing if by-products generated by wineries could become a potential source of polyphenols. Pulsed electric field and pectinase treatments were both effective in enhancing the extraction of polyphenols from grape pomace and peel. The extracts showed a strong antioxidant power.

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Table of Contents

List of tables

Page

List of figures

Page

Chapter 1: Introduction

Grapevine (Vitis spp.) cultivation is worldwide, yielding a wide range of products that take part in our daily diet. Viticulture is one of the major horticultural industries of the world with the area of grapevines exceeding 7.9 million ha (OIV, 2006). Polyphenols play an important role in wine making. By conferring color and astringency they contribute to the sensory properties of the wine. Beside their functional properties, their biological properties have been reported too. The dietary consumption of grape and its products is associated with a lower incidence of degenerative diseases such as cardiovascular disease and certain types of cancers. Moreover, grapes polyphenols possess many biological activities such as antioxidant, cardioprotective, anticancer, anti-inflammation, antiaging and antimicrobial properties (Xia E.Q. *et al*, 2010). Grape pomace is the residue remaining after the grapes have been pressed for wine-making which is generally composted and then used in soil conditioning or as cattle feed. It includes the skins, pulp, seeds and stems and constitutes around 20 % of the weight of the processed grapes (Bousetta N. *et al*, 2009). Grape pomace is a major source of phenolic compounds that were poorly extracted during winemaking, and anthocyanins, catechins, flavonol glycosides, phenolic acids and stilbenes are the principal phenolic constituents (Kammerer D. *et al*, 2004).

We present here two different techniques that we used and compared in terms of extracting polyphenols from grape pomace. In the first method we used pectinases which are the enzymes that degrade pectin, a polysaccharide found in the cell wall of plants (Whitaker R.J., 1994). In the second technique, we used a pulsed electric field which is a non thermal processing technology that is mainly used as a substitute for conventional thermal processing methods (Qiu X. *et al*, 1998)

Furthermore, by extracting bioactive compounds from low value products such as wine pomace, we can increase its value and generate additional income to farmers and wine makers. These bioactive compounds can be sold as polyphenol extracts, functional food components, health ingredients, and antioxidant additives.

Chapter 2: Literature review

Grapes: taxonomy and viticulture

Grapevine (Vitis spp.) cultivation is worldwide, yielding a wide range of products that take part in our daily diet. Viticulture is one of the major horticultural industries of the world with the area of grapevines exceeding 7.9 million ha. (OIV, 2006) Grapes were the fourth largest fruit crop produced in the world with around 66,935,199 tonnes produced in 2009. (FAOSTAT, 2010)

Most grapes are grown for wine production. When first discovered, the fruits were used for fresh consumption. Today, the fruit is used in a wide variety of products ranging from fresh fruits, to jams, juices and raisins. (Creasy L.G and Creasy L.L., 2009)

Grapevines are classified in the genus *Vitis*, family Vitaceae. Members of the Vitaceae show a climbing habit characterized by tendrils and inflorescences opposite the leaves. The genus is divided into 2 subgenera: *Euvitis* and *Muscadinia*. Most commercial grapes come from cultivars of *Vitis vinifera*, the grapevine native to the Mediterranean region. The rest, come from American and Asian species such as *Vitis labrusca*, *Vitis riparia* and *Vitis rotundifolia*. (Jackson S.R., 2008)

The cell structure of Grapes

The grape berry skin accounts for 6-9 % of the total berry weight. Compounds such as anthocyanins, tannins and aroma or their precursors are located within the skin cells.

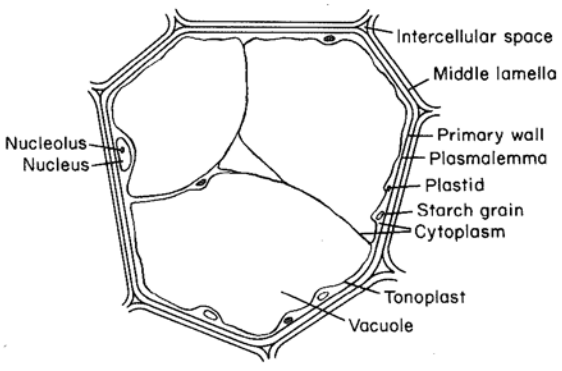


Figure 1.1: Idealized mature plant cell (Whitaker J.R., 1984)

Skin cells are surrounded by a thick pecto-cellulosic wall which provides rigidity to the berry and during winemaking it prevents the diffusion

of intracellular compounds into the must. The pulp represents 75-85 % of the ripe berry weight. It comprises large cells with fine pecto-cellulosic walls offering limited mechanical resistance. In the cell, the vacuole is a concentrated solution of organic acids, fermentable sugars, some aroma and precursors. Sugars and acids are concentrated in the flesh. The sugar content may reach as high as 28 %. The total acidity is mainly due to tartaric and malic acids which represent around 70 % of the total acids in the grape. Pectins which are galacturonic acid polymers of 30,000 to 40,000 MW are located between the cells, in the primary wall and lamella. Rhamnose, arabinans, galactans and arabinogalactans can also be included in pectic substances. (Whitehurst J.R. and Van Oort M., 2009) (Bamforth W.C., 2005) Therefore, it is obvious that the extraction of cellular components during wine making requires the degradation of the middle lamella wall to release the cells and the destabilization of the cell walls to allow the diffusion and extraction of the vacuole content. This is usually done by the use of one or the combination of the following agents: mechanical maceration, heat, pectinase enzyme...

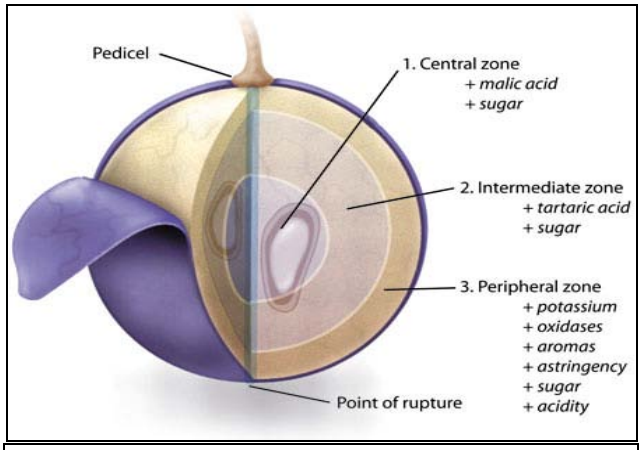


Figure 1.2: The three juice zones in the grape berry. (Dunsford P.A. and Sneyd T.N.,1989)

Grape pomace

Grape pomace is the residue remaining after the grapes have been pressed for wine-making. It includes the skins, pulp, seeds and stems. In general, the grape pomace constitutes around 20 % of the weight of the processed grapes (Bousetta N. *et al*, 2009). In Europe alone, wineries generate around 14.5 million tonnes of grape by-products every year (Makris P. D. and Boskou G., 2008). It is considered as an environmental problem and in most cases, the pomace is composted and then used in soil conditioning or as cattle feed.

However, these waste materials are known for being rich in bioactive compounds which are primarily polyphenols. These polyphenols encourage an alternative way to upgrade the pomace value. Some of the possible alternatives would be the production of polyphenol extracts, functional food components, health ingredients, and antioxidant additives. (Bousetta N. *et al*, 2009). Several methods have been proposed to enhance the extraction of polyphenols from grapes such as enzymatic techniques or electrical extraction. When grapes are processed into wine, it is inevitable that some of the phenolics will be leached into the liquid phase. However, an important portion will remain with the pomace, making it a valuable source of polyphenols that may have many applications as food and nutritional additives. (Bousetta N. *et al*, 2009)

Due to the high levels of phenolics in pomace, problems sometimes occur in germination of seeds in pomace mulched plots. In addition to that, when grape pomace is used as animal feed, it is poorly digested. As a result, alternatives to pomace for fertilizers and feed need to be found. (Negro C. *et al*, 2003) On the other hand, grape pomace is a major source of phenolics that were poorly extracted during winemaking.

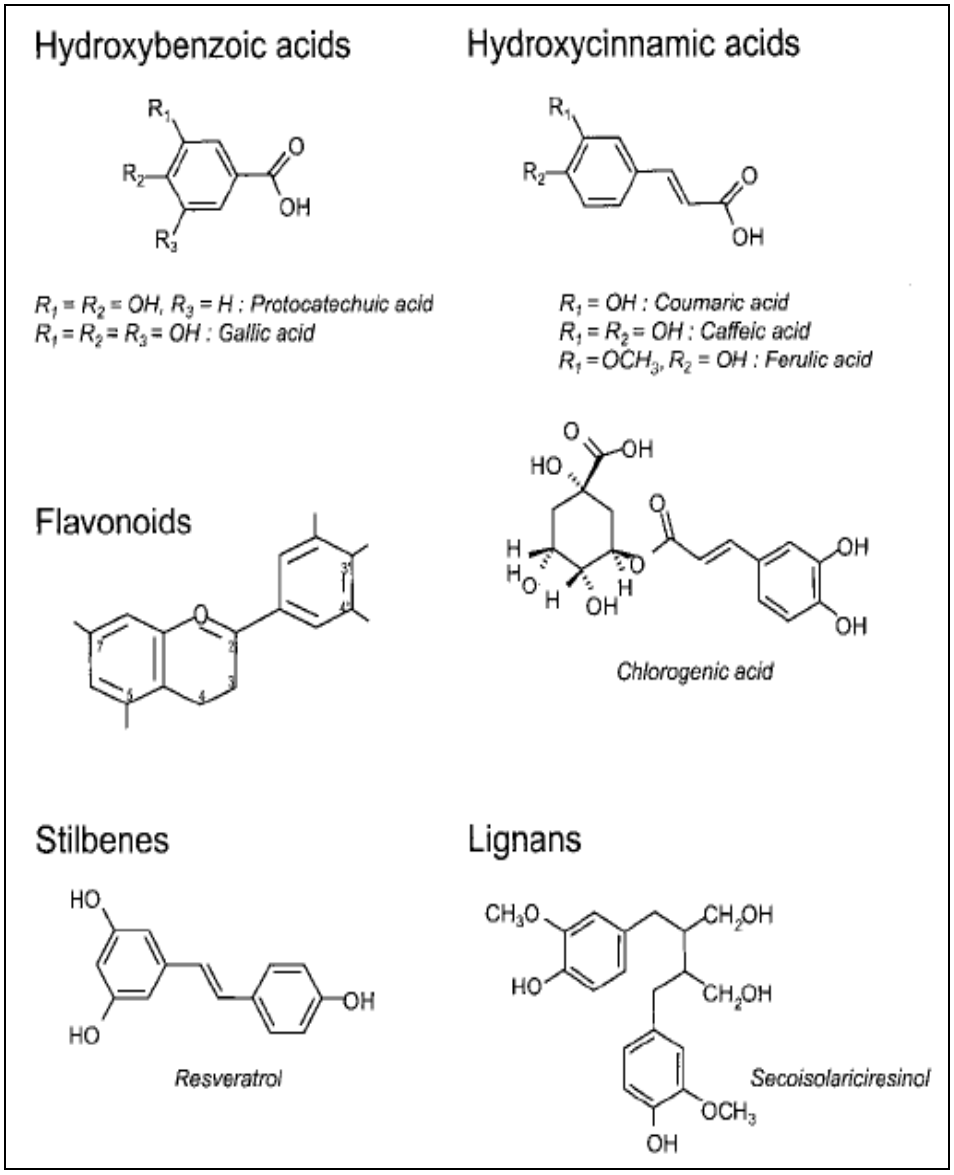
Anthocyanins, catechins, flavonol glycosides, phenolic acids and alcohols, and stilbenes are the principal phenolic constituents of grape pomace. Anthocyanins have long been extracted from the grape pomace and used as natural food colorants. In addition to that, grape pomace is a rich source of many high-value products such as ethanol, tartrates, malates, citric acid, grape seed oil, hydrocolloids and dietary fiber. (Kammerer D. *et al*, 2004)

Many of these compounds have many applications in the food industry. Therefore, finding a way to effectively extract some of these compounds could upgrade the value of the grape pomace and find additional applications for it. This will solve many of the environmental problems associated with the disposal of the pomace.

Polyphenol definition

The word “polyphenol” is formed from the Greek word poly meaning “many” and the word phenol which is a molecule formed by a phenyl (-C6H5) group bonded to a hydroxyl (-OH) group. Polyphenols are a major category of bioactive compounds known for their antioxidant activity and radical scavenging capacity. Phenolic compounds or polyphenols are the result of the secondary metabolism of plants. With more than 8000 structures, they represent one of the most widely distributed groups of compounds in the plant kingdom. From a structural point of view, phenolic compounds are characterized by having an aromatic ring bearing one or more hydroxyl substituent. It ranges from simple molecules such as phenolic acids to highly polymerized compounds such as condensed tannins (Shetty K. *et al*, 2006). (Fig.1.3)

Polyphenol in the plant kingdom



Plants produce a wide variety of polyphenols which function mainly as non signaling molecules such as attractors of pollinators, defense strategies, UV light protectors, and many more (Fraga G.C, 2009). However, it has been argued that the first polyphenols functioned as signaling molecules. For example, Lunularic acid, a stilbenoid, has been proposed to function in "lower" plants as a stress response hormone. In higher plants, a similar example of polyphenols functioning in regulatory roles is the flavones

induced pollen germination in petunia (Masoro J.E. and Austad N.S., 2006). Tannins are the most abundant polyphenols in the plant kingdom, found in nearly all families of plants, and comprising up to 50 % of the dry weight of leaves. (Bryan S.N., 2009)

Polyphenol classification

Depending on the number of phenol rings that they contain and on the structural elements that bind these rings to one another, polyphenols are classified into different groups. Thus, four groups can be distinguished: the phenolic acids, flavonoids, stilbenes and lignans.

Flavonoids consist of two aromatic rings (A and B) that are bound together by 3 carbon atoms forming a ring C, are subdivided into 6 subclasses: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (catechins and proanthocyanidins). In addition to all that, polyphenols can be linked with one another, or with various carbohydrates and organic acids (Manach C. *et al*, 2004) (Fig 1.4)

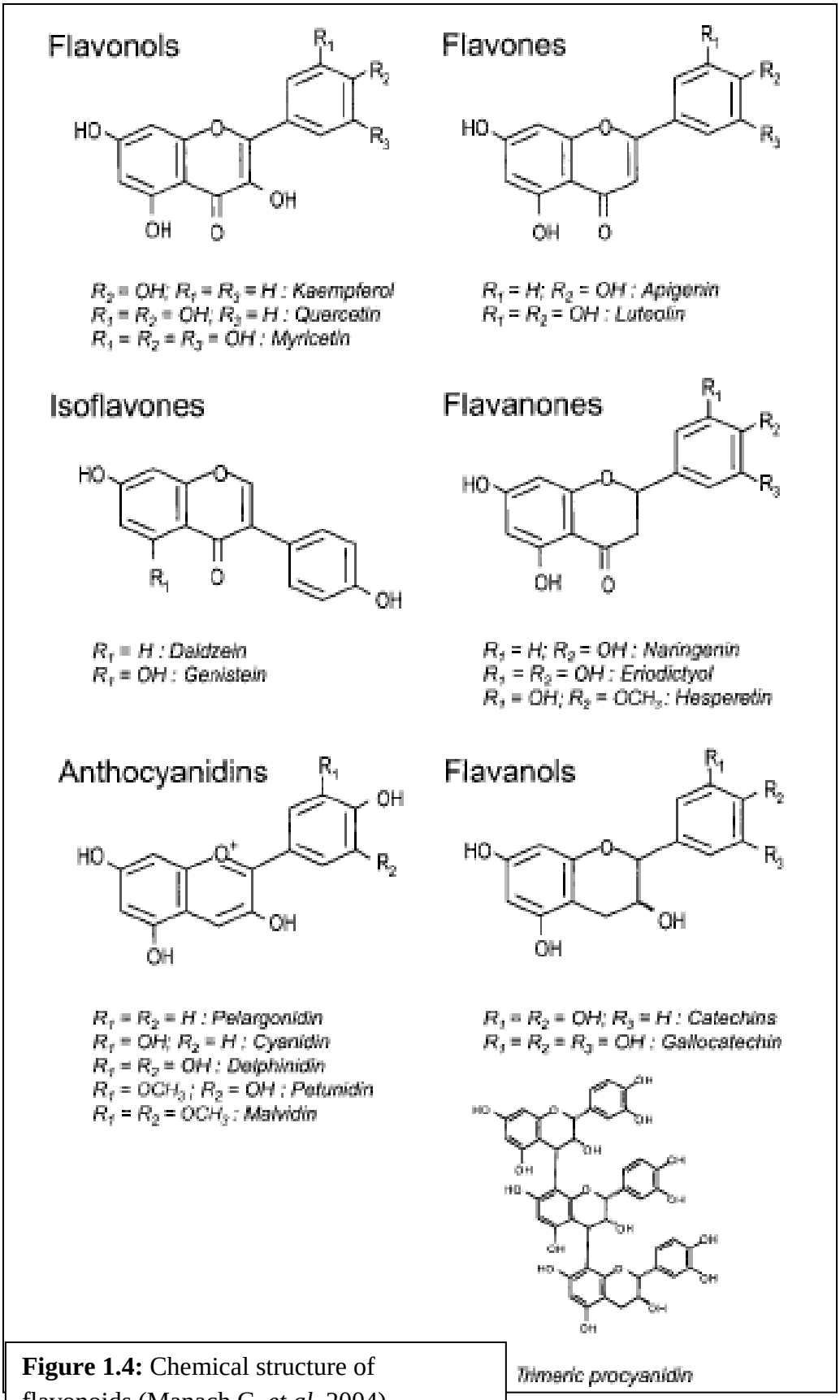


Figure 1.4: Chemical structure of flavonoids (Manach C. *et al*, 2004)

Polyphenol occurrence in food

Table 1.1: Polyphenol content of a typical serving of commonly consumed foodstuffs and beverages (mg) (Scalbert A. and Williamson G., 2000)

Foodstuff (Quantity)	Phenolic acids	Flavonols	Catechin monomers	Proantho-cyanidins	Flavanones	Anthocyanins
Potato (200 g)	28					
Tomato (100 g)	8	0.5				
Lettuce (100g)	8	1				
Apple (200g)	11	7				
Cherry (50g)	37	7	21	200		
Wheat bran (10g)	50	1	3	35		200
Dark chocolate (20g)			16	86		
Orange juice (100ml)					22	
Red wine (125 ml)	12	2	34	45		4
Coffee (200 ml)	150					
Black tea (200ml)		8	130			

In the human diet, the main sources of polyphenols come from fruits and beverages such as red wine and tea. Some polyphenols are found in many kinds of plant products, while others are limited to few species. For example, quercetin is found in all plant products (fruits, vegetables, leguminous plants, fruit juices, tea, wine…) whereas

flavanones are limited to citrus fruit, isoflavones to soya and phloridzin to apples (Manach C. *et al*, 2004).

Polyphenols in grapes

Grapes seeds and skins are an important source of phytochemicals such as gallic acid, catechin and epicatechin known for their anitoxidative capacity. Tannins and anthocyanins in grapes are responsible for the sensory properties of wine such as color and astringency (Yilmaz Y. and Toledo T.R., 2004).When grapes are processed into wine, it is inevitable that some of the phenolics will be leached into the liquid phase. However, an important portion will remain with the pomace, making it a valuable source of polyphenols that may have many applications as food and nutritional additives (Bousetta N. *et al*, 2009). In red wine, anthocyanins and flavonoids are the major two groups of phenolic compounds, and (+) - catechin is an abundant flavonoid (Bell J.R.C. *et al*, 2000).

Grape is a phenol-rich plant, and these phenolics are mainly distributed in the skin, stem, leaf and seed of grape, rather than their juicy middle sections. Total concentration of phenolic compounds was about 2178.8, 374.6, 23.8, and 351.6 mg/g GAE (gallic acid equivalent) in seed, skin, flesh, and leaf, respectively (Xia E.Q. *et al*, 2010). In another study that was done on 16 raisin grapes, the total phenolic content, determined in gallic acid equivalents using the Folin‑Ciocalteau assay, ranged from 316.3 to 1141.3 mg gallic acid/100 g DW (Breska *et al*, 2010). A study that evaluated the extraction techniques for phenol release, stated that the phenolic content of grape skin ranges from 285 to 550 mg phenols/kg of grape skin depending on the grape variety and type of pre-treatment (Pinelo M. *et al*, 2006)

The phenolic compounds mainly include anthocyanins, flavanols, flavonols, stilbenes (resveratrol) and phenolic acids. Anthocyanins are the red pigments responsible for the red color in grapes and wine. They are located in the first external layers of the hypodermal tissue and mainly in the vacuoles, as well as in special structures called anthocyanoplasts. The most important grape anthocyanins are the 3-glucoside forms of cyanidin, peonidin, petunidin, delphinidin and malvidin.

Flavonoids are widely distributed in grapes, especially in seeds and stems, and principally contain (+)-catechins, (−)-epicatechin and procyanidin polymers.

Flavonols are located in the solid parts of grapes, particularly in the skin and herbaceous parts and are mainly present as the 3-glycosides and 3-glucuronides of quercetin and myricetin, the 3-glucosides of kaempferol and isorhamnetin, in addition to laricitrin and syringetin which are predominantly found as 3-glucosides.

Catechins are located mainly in the seeds and skins. The major monomers are (+)-catechin, (–)-epicatechin and (–)-epicatechin-3-*O*-gallate (Xia E.Q. *et al*, 2010).

Table 1.2: The phenolic compounds in different parts of grape and its products (Xia E.Q. *et al*, 2010).

Source	Phenolic compounds
Seeds	gallic acid, (+)-catechin, epicatechin, dimeric procyanidin, proanthocyanidins
Skin	Proanthocyanidins, ellagic acid, myricetin, quercetin, kaempferol, trans-resveratrol
Leaf	myricetin, ellagic acid, kaempferol, quercetin, gallic acid
Stem	rutin, quercetin 3-O-glucuronide, trans-resveratrol, astilbin
Raisin	hydroxycinnamic acid, hydroxymethylfurfural
Red wine	malvidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, catechin, quercetin, resveratrol, hydroxycinnamic acid

Therefore, the grape and its products are important ingredients to be included in the human diet. This is because the majority of the phenolic compounds are known to have beneficial effects on the human health. These effects will be discussed later.

Polyphenol bioavailability and metabolism

The absorption and metabolism of polyphenols are still not well known. However, it is sufficient to state that some polyphenols are bioactive compounds that are absorbed in their native or modified form from the gut. Then they are metabolized with products detected in the plasma that retain a part of their antioxidant activity and they are excreted. Research has analyzed the plasma and urine of subjects with varying levels of polyphenols and antioxidants. (Okushio K *et al*, 1999a ; Okushio K *et al*, 1999b ; Morand C. *et al*, 1998) In humans, studies aim at identifying the compounds in their native form or their metabolites in the plasma and urine after the consumption of test meals or drinks. Most of the studies were based on the detection of quercetin after the consumption of onions, tea and apple juice (Mcanlis G.T. *et al*, 1999) (Lean M.E. et al, 1999).

Important findings have been found about the identification of various bioavailable polyphenols present in tea and wine, as well as their metabolites. A study done by Donovan et al, stated that the consumption of red wine leads to the plasma accumulation of methylcatechin which is a catechin metabolic product (Donovan J.L *et al*, 1999). Pietta et al, evaluated the absorption and metabolism of polyphenols present in green tea. They detected that around 15 % of the polyphenols administered will be present in the plasma and urine. These phenolic acids would result from bacterial metabolization of catechin and quercetin in the gut. The benzopyranosic ring is cleaved by the enzymes produced by the intestinal flora (Pietta P.G. *et al*, 1998)

Methylation is one of the metabolism reactions that occur in the liver and kidney. (Okushio K *et al*, 1999a) (Okushio K *et al*, 1999b). Polyphenols glucuronidation occurs in the intestine and the liver (Piksula M.K and Terao J., 1998)

Polyphenol extraction methods

There are three main techniques that can be used for the extraction of polyphenols from plant materials: Extraction using solvents, solid-phase extraction and supercritical extraction.

- Solvent extraction: Alcohols are known to provide the highest yields. The most widely used for extracting polyphenols are methanol and methanol/water mixtures. Other solvents such as acetone, ethyl acetate and solvent mixtures have been used, but they usually provide lower yields. Among the factors that influence the efficiency of the solvent extraction are the pH of the extraction medium and the temperature. The pH affects the degree of solubility of the compounds and influences the possible solubilization of the hydrolysable fraction. Concerning the temperature effect, it is known that heat makes the cell walls permeable, thus increasing the solubility of the polyphenols. (Flamini R., 2008)
- Solid phase extraction: This is a rapid and economical alternative to solvent extraction since it reduces the volume of organic solvent required. Extraction with C18 cartridges has been mostly employed for the extraction of phenolics from red wines, grapes, apples and other products. In this case, carbon loading and pore size are the determining factors affecting the separation efficiency. A higher pore size increases the retention capacity due to stronger interactions between the non-

polar surface and the analyte. A higher carbon loading leads to a greater retention of the ionized phenolic acids (Suarez B. *et al*, 1994).

- Supercritical fluid extraction: it combines the characteristics of gases and liquids for extraction. The low viscosity of the supercritical fluids confers a high capacity for diffusion and improves access to phenolic compounds bound to the cell wall. At the same time, it's high density provides a high solvation power which facilitates the extraction process. Supercritical carbon dioxide is the most widely used extraction solvent. (Flamini R., 2008)

Extraction of polyphenols from grape

Liquid-liquid extraction is usually used for the extraction of phenolic compounds from grapes. Either An alcohol or a hydroalcoholic solution are used. In most cases, the extraction solvent is ethanol, methanol, acetone or formic acid and water in different ratios. The use of solvents containing a mineral acid allows the extraction of all phenolic classes (Flamini R., 2008). For grape skin, the crude extract is rich in antocyanins and flavonols. Grape seeds are pressurized and heated in order to extract flavanols and hydroxycinnamic derivatives. In order to reduce or eliminate the use of organic solvents, many extraction methods were developed such as supercritical fluid extraction, microwave assisted extraction and ultrasound assisted extraction… (Xia E.Q. *et al*, 2010). Care must be taken in order to avoid degradation reactions such as hydrolysis, oxidations and polymerization (Flamini R., 2008).

Biological effects of polyphenols

The benefits of polyphenols were long discussed and researched. Polyphenols are well known for their antioxidant activity and due to that effect, they exhibit a wide range

of biological effects. Many studies confirmed that an excessive production of free radicals result in injuries such as cardiovascular diseases, some prenatal complications, neoplastic diseases, inflammatory state, Parkinson's disease, Alzheimer's disease, or ageing of the organism (Darlington L.G. and Stone T.W., 2001). The best way to prevent these diseases is the consumption of an optimal diet containing natural antioxidants. Currently, researchers have been discovering the importance of polyphenolic compounds present in plants, which not long ago were considered unnecessary for the human diet. (Cieslik E. *et al*, 2004)

The antioxidant activity is the most notable bioactivity of phenolic compounds from grapes. Some of the observed actions include scavenging of free radicals, inhibition of lipid oxidation and reduction of hydroperoxide formation…They inhibit LDL oxidation in vitro. In fact, LDL isolated from volunteers supplemented with red wine or red wine polyphenols showed a reduced susceptibility to oxidation (Nigdikar S.V. *et al*, 1998). They also protect DNA from oxidative damage with important consequences in the age-related development of some cancers (Halliwell B., 1999). Another example is (+) – Catechin which exhibitis some antioxidant activity in the human plasma (Masoro J.E. and Austad N.S., 2006). The antioxidative activity of phenolic compounds is mainly attributed to their free radical scavenging and metal chelating properties. In addition to that, they exhibit an effect on cell signaling pathways and on gene expression (Soobrattee M.A *et al*, 2005) (Dell Agli M. *et al*, 2005). In flavonols, the number and the position of the OH group on the ring determined the antioxidant capacity (Xia E.Q. *et al*, 2010).

Another important effect on human health is a cardioprotective action. One of the classic examples is a phenomenon known as the "French Paradox". This paradox is

attributed to wine phenolics and especially resveratrol. It refers to some observations in France where a high consumption of wine reduced the risk of coronary heart diseases despite a diet rich in saturated fats (Meyer S.A. *et al*, 1998). Postprandial hyperlipemia and oxidative stress, a well-defined risk factor for atherosclerosis, could be reduced by grape seed extracts or phenolic-rich grape juice. Anthocyanins from wine and grape skin inhibited phosphodiesterase-5 activity, which reduced the risk of cardiovascular diseases by vasorelaxation. Red wine consumption reduced oxidative stress induced by Cu-oxidised LDL and increased HDL cholesterol concentrations. Grape phenolics showed beneficial effects in regulating the plasma lipid and oxidative stress (Xia E.Q. *et al*, 2010).

Several types of polyphenols (phenolic acids, hydrolysable tannins, and flavonoids) show anticarcinogenic and antimutagenic effects. Polyphenols might interfere in several of the steps that lead to the development of malignant tumors, inactivating carcinogens, inhibiting the expression of mutant genes and the activity of enzymes involved in the activation of procarcinogens and activating enzymatic systems involved in the detoxification of xenobiotics (Bravo L., 1998). A study done by Hudson *et al* reported that the grape skin extract induced prostate tumor cell lines apoptosis with high rates. In addition to that, it was proven that wine by-product would help to fight against carcinogenesis. In fact, the extract from pomace remaning after wine production expressed a significant antiproliferative effect on human colon adenocarcinoma cells (Lazze M.C. *et al*, 2009) (God J.M. et al, 2007). Resveratrol inhibits DNA damage induced by Reactive oxygen species (ROS) and therefore exhibits a protective action against cancer (Xia E.Q. *et al*, 2010).

Studies on rats and human confirmed the anti-inflammatory effects of grapes phenolic compounds. This effect can be possibly attributed to the flavonols, flavanols and procyanidins. (Chacona M.R. *et al*, 2009) (Terra X. *et al*, 2009). By inhibiting inflammation at mRNA levels, procyanidins in grapes resulted in major beneficial health effects such as decreasing the risk of diseases linked to high fatty diets and obesity, such as cardiovascular and metabolic disorders (Xia E.Q. *et al*, 2010).

Polyphenols might be beneficial in reversing the course of neuronal and behavioral aging. They prevent organs and tissues from oxidative damage. This is due to their antioxidant potential such as scavenging free radicals. Further research discovered that supplementing with grape seed extracts for 30 days, inhibited the accumulation of age-related oxidative DNA damages in the neural tissue (Balu M. *et al*, 2006)

Phenolics have been described as antimicrobial agents demonstrating antibacterial, antifungal and antiviral effects (Xia E.Q. *et al*, 2010). Alcohol-free red and white wine extracts exhibited antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. This suggests that polyphenolic compounds in red wines were responsible for the antimicrobial effects (Papadopoulou C. *et al*, 2005) The antimicrobial activity of fermented pomace was either as effective as or significantly better than whole fruit grape extracts. The phenolic compounds from different parts of grapes displayed different antimicrobial effects (Thimothe J. *et al*, 2007). Brown *et al*, showed that the antimicrobial activity against *Helicobacter pylori* increases in this order: flesh, whole fruit grape extracts, fermented pomace, skin, leaves and seed. Resveratrol in grapes exhibited a powerful antifungal activity against the human pathogenic fungus *Candida albicans* (Brown J.C. *et al*, 2009). The antimicrobial activity of phenolic

compounds might be dependent on the number of hydroxyls and the degree of polymerization (Xia E.Q. *et al*, 2010).

Table 1.3: Bioactivities of some phenolic compounds from grapes (Xia E.Q. *et al*, 2010).

Phenolic compound	Bioactivity
Resveratrol	Free radical scavenging Antiproliferation Enhancing plasma NO level Regulating lipid metabolism Protection against membrane oxidation
Quercetin	Antibacterial Enhancing plasma NO level
Catechin	Anticancer Free radical scavenging Antibacterial Anti-inflammation Protection against membrane oxidation
Flavone	Antiproliferation
Flavonol	Free radical scavenging
Procyanidin	Anticancer Free radical scavenging Anti-inflammation Antioxidant
Anthocyanin	Vasorelaxation Free radical scavenger Antibacterial Antioxidant Induced apoptosis
Gallic acid	Free radical scavenger
Epicatechin	Antibacterial

Polyphenol applications

Commercial applications of anthocyanins as food colorants include soft drinks, fruit preserves (jams, canned fruit), sugar confectionary (jellies), yogurt, dry mixes (acid

dessert mixes and drink powders) and a few alcoholic drinks. Soft drinks have been the main and ideal target for use of anthocyanins as a colorant. The problem with the use of anthocyanins as food colorants resides in their instability in acid food matrixes. However, grape extracts have proved to be a successful candidate to be used in foods for two reasons: the first one is that anthocyanins may be easily obtained in high quantities from grapes and the second one is that grape anthocyanins are more stable towards pH variations and in the presence of SO_2 (Gould K. *et al*, 2008).

It is clear that polyphenols have many beneficial effects on human health. Therefore, they can be sold as supplements for the human diet to help in the prevention of degenerative diseases particularly cardiovascular diseases and cancers (Scalbert A., *et al*, 2005).

The application of phenolic compounds as natural preservatives and antimicrobial agents for food is very promising. Sivarooban et al. found that phenolic compounds can be used in ready-to-eat food products in order to maintain shelf life and improve safety (Sivarooban T. et al, 2008).

Enzymes

Enzymes are natural proteins that act as very effective catalysts by increasing reation rates by many orders of magnitude. They are specific to a single substrate and to a single reaction direction. Enzymes act as very effective catalysts, increasing reaction rates by many orders of magnitude. Enzymes are active organic substances secreted by cells, and have the property under certain conditions of facilitating chemical reactions without entering into the composition of the definite products which result. Most

enzymes have clear temperature and pH optima, and their action can often be inhibited or enhanced by certain other compounds or co-factors (Palmer T. and Bonner L.P., 2007).

Pectic enzymes

The pectic substances are constituents of cell walls and of intercellular layers of all higher plants, along with cellulose and hemicelluloses. These substances are also found in juices and saps and contribute to the texture of fruits such as tomatoes and citrus. Pectins have a linear α-1,4 linked chain of pyranosyl D-galacturonic acid molecules, which is referred to as the polygalacturonan, or galacturonan backbone. These galacturonic acid molecules are often esterified with methanol, and if the degree of methylation is greater than 50% it is referred to as "high methoxy pectin". Grape pectins are 44-65% esterified (Lanzarini G. and Pifferi P.G., 1989). Cellulose is a linear chain of β-1,4 linked glucose molecules. Hydrogen bonding between these chains occurs, resulting in microfibrils with varying degrees of crystallinitiy. Cellulase is a system of enzymes comprising endo-glucanase, exo-glucanase and cellobiase (β-glucosidase) which will hydrolyse the cellulosic backbone. Hemicellulose is a polysaccharide containing four units: arabinans, galactans, xylans and xyloglucans. Arabinases and galactanases enzymes hydrolise the hemicellulosic backbone (Rose K.C.J., 2003)

Pectic enzymes are the enzymes that will degrade pectin, a polysaccharide found in the cell wall of plants. They are important to food scientists because they are used in treating fruit juices and beverages to facilitate filtration and clarification as well as to increase juice yields. They are the reason of softening of many fruits and vegetables during ripening and in that case they are considered as deteriorative enzymes.

Pectic enzymes include pectinesterase, polygalacturonases and pectate lyases, all of them specific to methyl D-galacturonic acid and D-galacturnoic acid units of rhamnogalacturonans. In addition to that, pectic enzymes also include α-L-arabinofranosidase and endo-arabinase, both of them acting on arabinans (Whitaker R.J., 1994)

Therefore, pectinases are often used to weaken the cell wall and help in the extraction of some cellular compounds. These pectinases preparations are usually made up of polygalacturonase, pectinesterase, xylanase, hemicellulase and cellulase.

Pulsed electric field

Pulsed electric field is a non thermal processing technology that may arise as a substitute for conventional thermal processing methods (Qiu X. *et al*, 1998). The antimicrobial inactivation properties were first studied (Qin B.L. *et al*, 1995) PEF has the potential to be used to reduce pathogen levels in food while increasing the shelf life and retaining the nutritional properties (Dunn J., 1996)

Recently, it took another dimension due to its potential of breaking the cells and making it easier to extract natural components. Semi-industrial systems for the continuous PEF treatment are available now for sugar beets, apples and grape mash. Grape skin polyphenols bind in different ways and consequently, they are classified as cell-wall polyphenols which are bound to polysaccharides, and non cell-wall polyphenols which are present in the vacuoles and cell nucleus. PEF will provoke membrane electroporation and biological tissue damage. This will cause pore formation in the cell

membranes, and depending on the intensity and duration of the the electrical treatment, these pores can be reversible or irreversible (Bousetta N. *et al*, 2009).

PEF consists of applying pulses of high voltage to a material placed between two electrodes. Therefore, it damages the cell wall making it easier and faster to extract cell components. An experiment conducted at the University of Nebraska-Lincoln showed that PEF enhanced the extraction of anthocyanins from red cabbage by 2.12 times (Gashovska T.K. *et al,* 2006). In another study, polyphenol extraction from grape skins was increased after application of a pulsed electric field (Bousetta N. *et al*, 2009).

High pressure liquid chromatography (HPLC) separation of polyphenols

In order to study polyphenols and understand their function, it is necessary to find analytical techniques to separate, identify, and quantify them. High performance liquid chromatography (HPLC) emerged as a powerful technique used to separate and quantify polyphenols. . For a better investigation of these compounds, HPLC is often coupled with either a photodiode array detector or a mass spectrometer.

HPLC of polyphenols is most commonly done on the basis of a reverse phase (RP) chromatography, indicating a non-polar stationary phase and a polar solvent. In most cases, a C_{18} polymer bound to a silica support constitute the stationary phase. A small particle size of about 4-5 µm is used to ensure a high number of theoretical plates and therefore a good efficiency and resolution (Santos-Buelga C. and Williamson G., 2003) A gradient mobile phase based on water, an acid and an organic solvent are usually used. Acidification is necessary to prevent ionization of the acid groups of the phenolics.

The acids commonly used are phosphoric, formic and acetic acids (Flamini R., 2008) (Somers T.C. and Verette E., 1988)

Chapter 3: Materials and methods

3.1-Materials

Plant materials

The following grape cultivars were used in this study: Flame seedless (*Vitis vinifera* L.), St.Croix, Frontenac, and St.Pepin (*Vitis spp.*). Flame seedless grapes, imported from Chile, were purchased from a retail store (SuperSaver, Lincoln NE) on 17 May 2010. The pomace of St. Croix, Frontenac and St.Pepin, was sourced from James Arthur Vineyards, a local winery in Lincoln, NE. The pomace was collected immediately after pressing the berries harvested at their optimum maturity. St Croix was collected on 1 September 2010, St Pepin on 7 September 2010 and Frontenac on 8 September 2010. Samples were randomly collected from different locations in the batch and sealed in plastic bags. Samples were collected in triplicates and transported to the University of Nebraska, Lincoln where they were kept in cold storage (4°C) overnight until the next day when they were analyzed.

Chemicals

- Methanol ChromasolV, quercetin, trans-resveratrol, (-)-epicatechin, myricetin, ferric chloride, TPTZ, and ammonium iron (II) sulfate hexahydrate were purchased from Sigma-Aldrich.
- Catechin, gallic acid, acetic acid glacial and the pectinase 5KU from *Aspergillus niger* were purchased from MP biomedicals.
- Rutin was purchased from VWR.
- Acetonitrile ACS grade was purchased from Fisher Scientific
- Mixed bed exchange resin IONAC NM-60 H^+/OH^- form type I beads (16-50 mesh) was purchased from Baker analyzed.

- Pectinase 5KU, *Aspergillus niger* from MP biomedicals
- Folin reagent from Sigma-Aldrich
- Sodium acetate and sodium carbonate from Fischer scientific

Laboratory materials

- Waters Spherisorb ODS2 Column, 5 µm (250mm X 4.6 mm)
- Waters in line guard cartridge holder kit
- Waters Spherisorb ODS2 Guard column
- Waters peek one piece fingertight fitting
- 15 ml conical centrifuge tubes from Bio-Rad
- Acrodisc 13 mm syringe filter with 0.2 µM nylon membrane
- BD 3 ml syringe with luer-lok tips from Fischer Scientific
- Conical glass insert 200 µl from Fischer Scientific
- 8 MM TEF/SIL septa from Fischer Scientific
- Whatman filter paper number 4
- 0.45 µM Whatman filter disks
- 1000 – 200 µl micropipettes
- Laboratory glassware: Erlenmeyers, beakers, graduated cylinders, test tubes…

Instruments

- HPLC: Dionex Ultimate 3000 system, equipped with a Dionex Ultimate 3000 pump, Dionex Ultimate 3000 photodiode array detector, Dionex Ultimate 3000 column oven, Dionex Ultimate 3000 autosampler.
- Shimadzu UV Spectrophotometer, UV 1800

- PEF : Hipotronics discharger model CF 60/25-12C
- General Atomics high voltage capacitor
- Beckman GS-15 R centrifuge
- Magnetic stirrer
- Water bath

3.2-Methods

Sample preparation

Grape skins: the Chilean Flame grapes were peeled manually. The flesh was discarded.

Grape pomace: prior to any treatment application, the pomace was ground using a domestic food professor to homogenize the skins, seeds and stems.

Treatments

PEF treatment: Around 20 g of sample were introduced in the PEF chamber. The sample was pressed to eliminate air gaps. The following conditions were applied: 5 KV, 1 µFarad, 20 Pulses. The electric field strength was 2.5 kV/cm. The pulse frequency was 1 Hz. The pulse length was 2.7 seconds. The number of pulses was chosen according to a preliminary study (results and discussion). Samples were treated in triplicate.

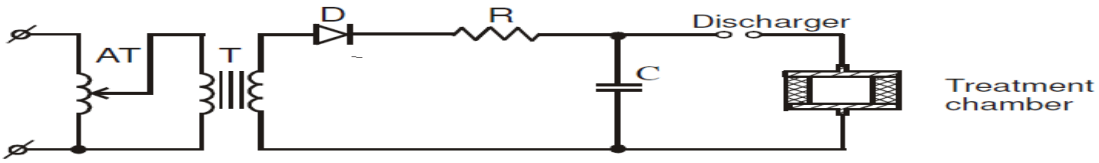


Figure 3.1: Electrical circuit diagram for the pulsed electric field generator (Gashovska T.K et al, 2006)

Enzymatic treatment: Pectinase 5 KU was used to break down the cell wall. According to the supplier recommendations, the optimal enzymatic activity is noticed at a temperature of 25 °C and a pH=4. For enzymatic hydrolysis 5g of samples were incubated in 50 ml of 0.1 M acetate buffer pH 4.0 for 2 hours and a temperature of 25°C. Enzyme/substrate ratio of 10 % corresponding to enzyme concentrations from 1% of the total reaction volume was used (Meyer S.A. *et al*, 1998). The duration of incubation was determined according to a preliminary study (results and discussion). Samples were treated in triplicate.

Polyphenol extraction

PEF treated samples: 5g of treated and non treated samples were added into separate 250 ml volumetric flask. 5g of non treated samples that were not exposed to the PEF treatment will serve as the control. Distilled water was used as the extraction solvent. For this purpose, 50 ml of water is added into each volumetric flask. Then the flasks are placed in a water bath at 50 °C for 1 hour. The temperature and the duration of the water bath treatment were chosen according to a preliminary study (results and discussion). After that, the extract was filtered and collected into 250 ml amber glass bottles. The extract was frozen at -20 °C before undergoing further analysis.

Enzymatic treated samples: A solution of acetate buffer was prepared and adjusted to a pH = 4. Then, this solution was mixed with pectinase enzymes to achieve a concentration of 1%. A volume of 50 ml of that solution was mixed with 5 g of sample into a 250 ml volumetric flask. The control treatment was prepared by mixing 5 g of sample with 50 ml of acetate buffer not containing the enzymes. The flasks were

incubated for 2 hours in a water bath at a temperature of 25 °C. The extract was then filtered and frozen for further analysis.

Analysis

Total polyphenols test: (Folin-Ciocalteau method)

This test was done according to the following procedure:

- Dilute the red grape extract 10 times. The white grape extract is not diluted
- Add 1 ml of each sample into a 10 ml test tube.
- Prepare 2 blanks by adding 1 ml of distilled water in separate tubes.
- Add 1.25 ml of Folin reagent (previously diluted 10 times) to each tube.
- Let the tubes stand for 5 mins.
- Add 1 ml sodium carbonate solution (5 %) to the tubes.
- Close the tubes and incubate in a water bath at 45 °C for 15 mins.
- Spectrophotometer at λ= 765 nm.

The Standard curve was established by using Gallic acid (0-1-2-3-4-5 mg/100 ml)

Antioxidant test: FRAP: Ferric reducing antioxidant power

The FRAP antioxidant test was performed according to the following steps:

- Prepare a 300 mM solution of sodium acetate. Adjust the pH to 3.6
- Prepare 10 mM TPTZ (2,4,6-tripyridyl-2-triazine) in 40 mM HCl.
- Prepare 20 mM $FeCl_3$ solution in distilled water
- Make 100, 200, 400, 600, 800, 1000 μM solutions of ferrous ammonium sulfate for making the standard curve.

- Make the FRAP reagent by mixing acetate buffer, TPTZ and $FeCl_3$ in the ratio of 10:1:1
- Add 100 µL of sample or standard solution into a test tube
- Add 900 µL of FRAP reagent
- Wait 5 mins then read the absorbance at λ=593 nm

High pressure liquid chromatography HPLC:

All samples were filtered using a BD 3 ml syringe equipped with a 0.2 µM syringe filter. Samples that were subjected to the pectinase treatement were deionized by adding ion exchange resin beads. This allowed a better and more effective HPLC separation.

The HPLC analysis was performed on a Dionex Ultimate 3000 system, equipped with a Dionex Ultimate 3000 pump, Dionex Ultimate 3000 photodiode array detector, Dionex Ultimate 3000 column oven and a Dionex Ultimate 3000 autosampler. The results were analysed using chromeleon software (Version 6.80 SP1 build 2238)

Chromatography was conducted on a Waters Spherisorb ODS2 Column, (250mm x 4.6 mm, 5 µm particle size) from Waters. Separation was done at a column temperature of 30 °C and a flow rate of 1 ml/min. The injection volume is 20 µl. The method was based on the procedure used by 'Breksa et al, 2010' with some modifications. For the mobile phase a binary gradient was used: (A) water with 5 % acetic acid and (B) acetonitrile with 5 % acetic acid. Mobile phase solutions were filtered by passing them through a 0.45 µM filter prior to usage. The gradient programme used was the following: 3 min isocratic elution step with 5 % B, followed by 12 min linear gradient from 5 % to 9 % B, 7 min linear gradient to 13.5 % B, 20 min linear gradient to 18.5 % B, 6 min

isocratic elution with 18.5 % B, 3 min linear gradient to 22.5 % B, 4 min isocratic elution with 22.5 % B, 1 min linear gradient to 30.9 % B, 1 min linear gradient to 40.0 % B. At the end, the composition was brought to the initial conditions 5 % B and left for 10 mins before the next injection.

The following standard solutions were prepared by dissolving them in methanol to achieve different concentrations and to establish the standard curve: gallic acid, rutin, trans-resveratrol, catechin, myricetin, (-)- epicatechin and quercetin.

Statistical Analysis

All experiments were repeated three times and the data were analyzed using the Proc Mixed procedure of the Statistical Analysis System (Version 9.2 by SAS Institute Inc., Cary, NC). The 18 experimental units are outlined in Figure 3.2. Since there are multiple experimental units per treatment in each block, the experimental design is considered to be a generalized randomized complete block design (GRCBD). Results are presented as the means of the replicated treatments.

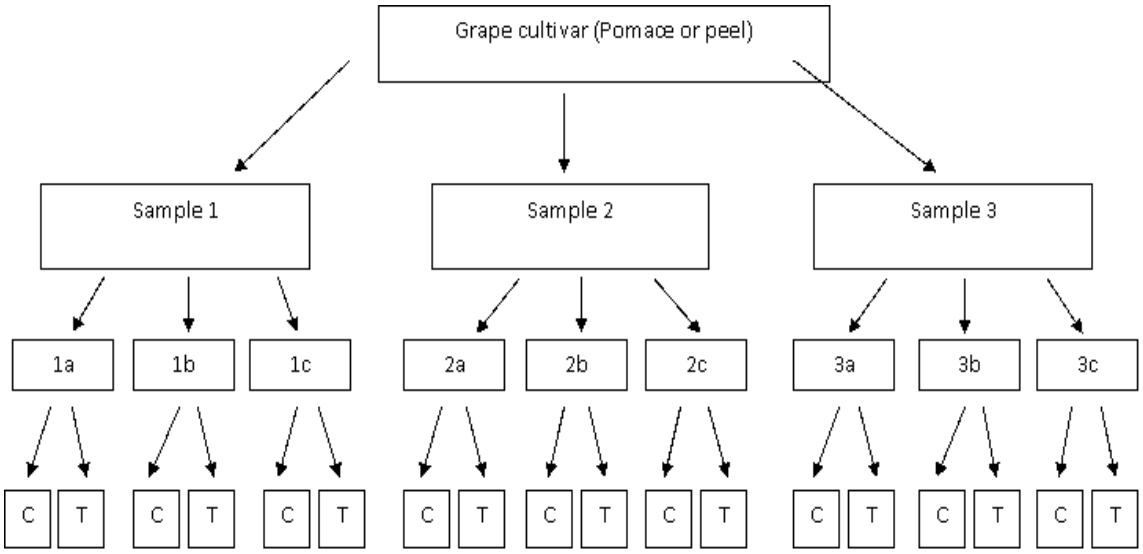


Figure 3.2: Experimental design for the PEF and pectinase treatments

Chapter 4: Results and Discussion

4.1- Preliminary study

Number of pulses

The number of pulses to be applied was determined by measuring the impedance. The impedance is the resistance of the cells to the passage of the electrical current. When the impedance ratio becomes constant, this indicates that the cells have been fully damaged.

This study was done according to the following steps:

- Apply the following number of P.E.F pulses (0 – 5 – 10 – 15 – 20 – 25 – 30 – 35 – 40 – 45 – 50 – 55 – 60) to the sample placed in the P.E.F chamber
- For every number of pulses applied, measure the impedance by placing the 2 electrodes of the impedometer on both sides of the P.E.F treatment chamber.

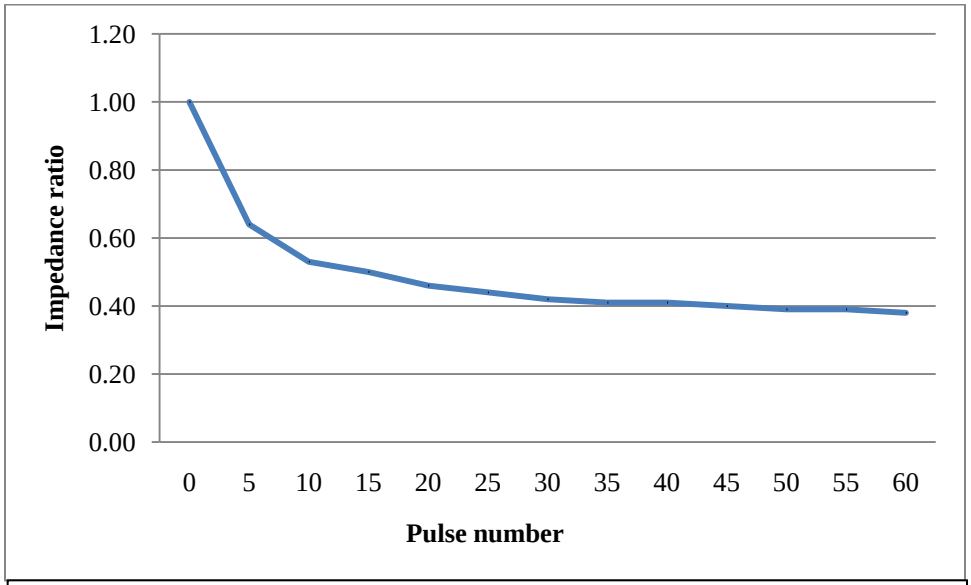


Fig 4.1: Variation of the impedance ratio over the number of pulses for the Flame peel

At 20 pulses, the impedance ratio becomes constant (Fig 4.1). This indicates that there is no point of applying more than 20 pulses because the cells are damaged. This is

also confirmed by a statistical analysis (Appendix C). There was no significant decrease in the impedance value when we applied more than 20 pulses. ($p = 0.3175$)

Duration of water bath

In order to determine the optimal duration of the water bath for the extraction of polyphenols, we incubated the samples in 3 different temperatures: 30 – 60 – 90 minutes. After that, the total polyphenols were analyzed by the Folin-Ciocalteau method. The results were statistically analyzed by the SAS software. The incubation of 60 minutes yielded the highest and statistically significant concentration of total polyphenols. (Appendix C)

Temperature of water bath

The optimal water bath temperature for extracting the polyphenols was determined by measuring the concentration of total polyphenols extracted from 5 g of sample incubated at the following temperatures: 30 – 40 -50 – 60 – 70 °C.

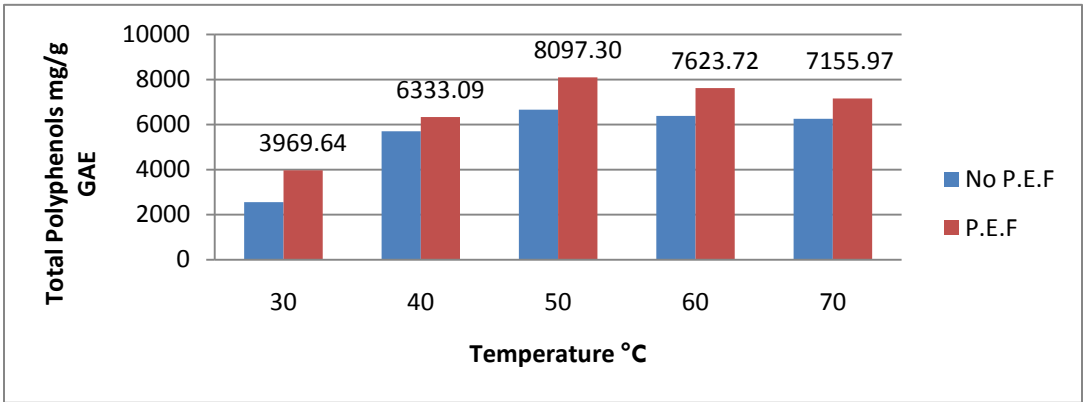


Fig 4.2: Temperature's effect of water bath on the concentration of TPP for the Flame peel

A temperature of 50 °C gave the highest concentration of TPP. (Fig 4.2) The results were analyzed by using SAS software. (Appendix C).

Duration of pectinase incubation

The pectinase enzymes were incubated for 1, 2 and 3 hours with the P.EF treated and non treated Flame peel. After that, the polyphenols were extracted, and their concentration determined. An incubation of 2 hours proved to be the most optimal. There were no significant difference between an incubation of 2 and 3 hours for both the treated samples ($p = 0.2665$) and the non treated samples ($p = 0.5753$) (Appendix C)

4.2- Total Polyphenols analysis

PEF treated samples

The TPP concentration was higher than the control in all samples that were subjected to a pulsed electric field treatment. Therefore, there was a significant effect ($p<0.05$) of the PEF treatment on the extraction of polyphenols from both pomace and peel. For the peel of the Flame cultivar, the treated sample showed a polyphenol concentration that is 1.2 times higher than the control treatment. In the case of the pomace, St.Croix was the richest in polyphenols, followed by Frontenac and St.Pepin. The PEF treatment increased total polyphenol extraction by 1.3 times in St.Croix, 1.2 times in Frontenac and 1.1 times in St.Pepin.

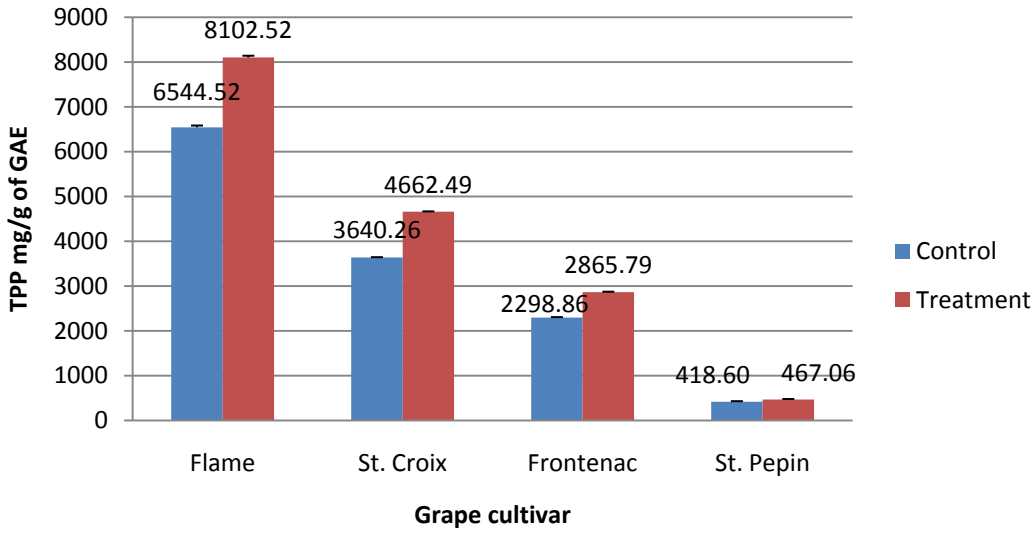


Figure 4.3: Concentration of TPP extracted from pomace and peel subjected to a PEF

The pomace of St. Croix was the richest in polyphenols with an average of 4662 mg/g of GAE in the treated sample. In fact, St Croix is the only cultivar that has a red juice. This explains its richness in polyphenols. St.Pepin, which is a white grape cultivar,

gave the lowest polyphenol concentration among the three cultivars. The peel of the Chilean flame cultivar yielded a high polyphenol concentration, even richer than the pomace. This can be explained by the fact that a lot of polyphenols are leached with the juice during pressing of the grape berries. At the end of the pressing, the pomace will retain a fraction of the polyphenols. However, during this experiment, grapes were peeled manually conserving their total polyphenol concentration. This explains why the peel of the Flame grapes gave the highest results.

Pectinase treated samples

All samples that were subjected to the enzymatic treatment resulted in a higher total polyphenol concentration than the samples that were not incubated with the enzymes. There was a significant difference ($p < 0.05$) between the control and treated pomace and peel. When the peel of the Flame cultivar was incubated with pectinase, it resulted in a TPP concentration that is 1.2 times higher than the control treatment. Similar results were obtained with the enzymatically treated pomace. Compared to the control, the pectinase treatement increased TPP extraction by 1.5 times for Frontenac, 1.1 times for St Croix and 1.3 times for St.Pepin.

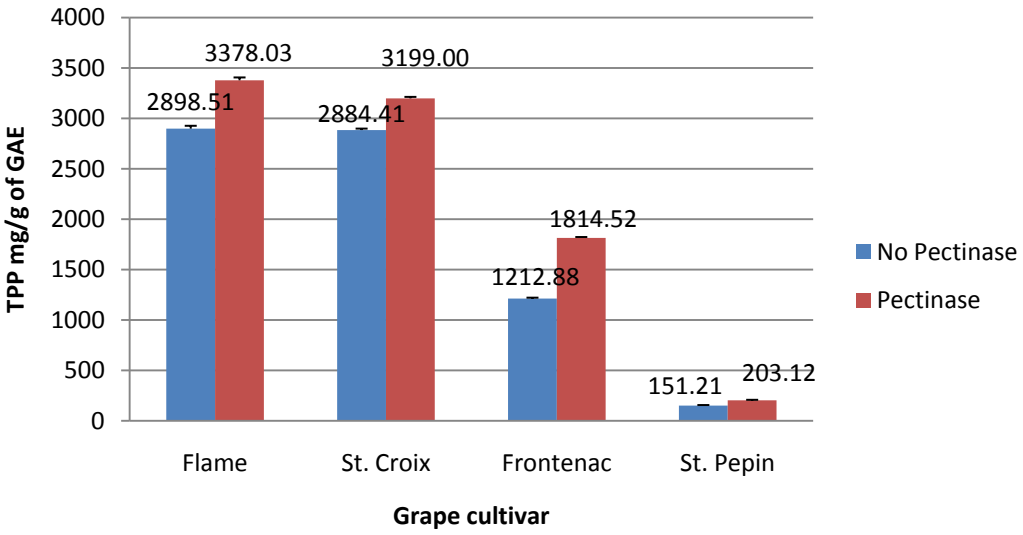


Figure 4.4: Concentration of TPP extracted from pomace and peel subjected to a pectinase treatment

The results were in agreement with the pulsed electric field results mentioned previously. The highest concentration of TPP was extracted from St.Croix, followed by Frontenac and last by St.Pepin. (fig. 4.2).

Both treatments were effective in improving the extraction efficiency of polyphenols from both grape pomace and peel. However, the pulsed electric field was more effective than the pectinase treatment. (fig. 4.3) When samples were subjected to a PEF treatment, they resulted in a higher TPP extraction than when they where incubated with pectinases.

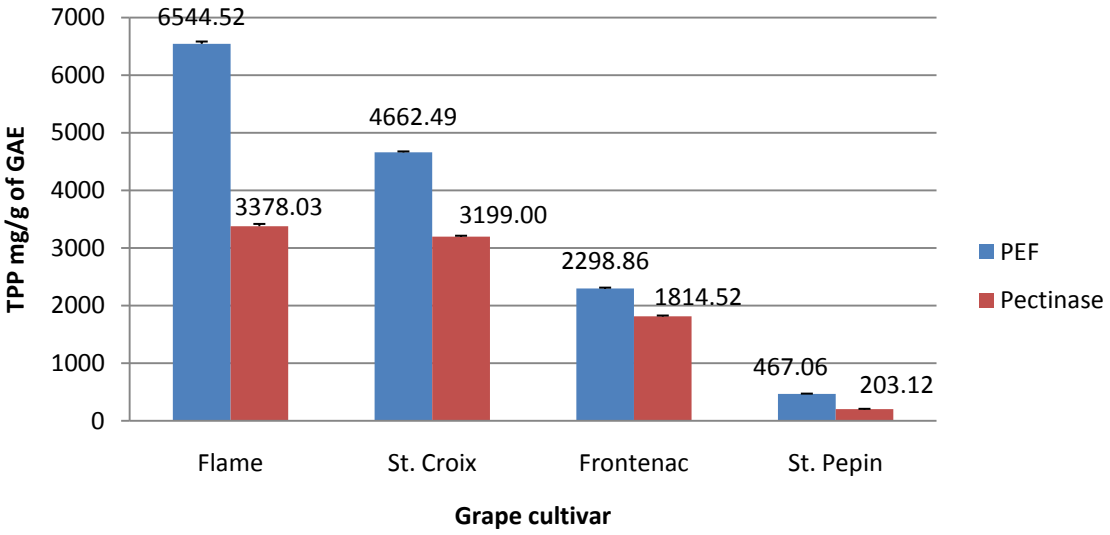


Figure 4.5: Comparison between the PEF and pectinase assisted extraction of TPP

The biggest difference was observed in the Flame grape peel where PEF was 2.4 times more effective than the enzymatic treatment. For St. Croix, the TPP concentration was 1.5 times more when the pomace was treated with a pulsed electric field than with pectinase. For Frontenac pomace, the TPP concentration was 1.6 times higher for the PEF treatment. And, in the case of St. Pepin, the electric field was 2.3 times more effective than the pectinase treatment in destabilizing the cells and facilitating the extraction of polyphenols.

4.3-HPLC analysis

Flame grapes peel

PEF treated samples

The following phenolics were identified in the extract of the flame grape peel that was subjected to a PEF treatment: gallic acid, catechin, rutin and myricetin. The concentration of these phenolics was higher in the treated samples than in the control. Therefore, the application of the pulsed electric field was effective in increasing the

amount of individual phenolics in the extract. Gallic acid extraction increased by 4.86 times in the treated samples, catechin by 1.35 times and rutin by 1.75 times. Myricetin was not identified in the control treatment. However, about 26.20 µg/g on average of myricetin were identified in the treated sample. Thus, the application of the electrical treatment allowed for the extraction of myricetin from the skins and consequently, the quality of the extract was improved.

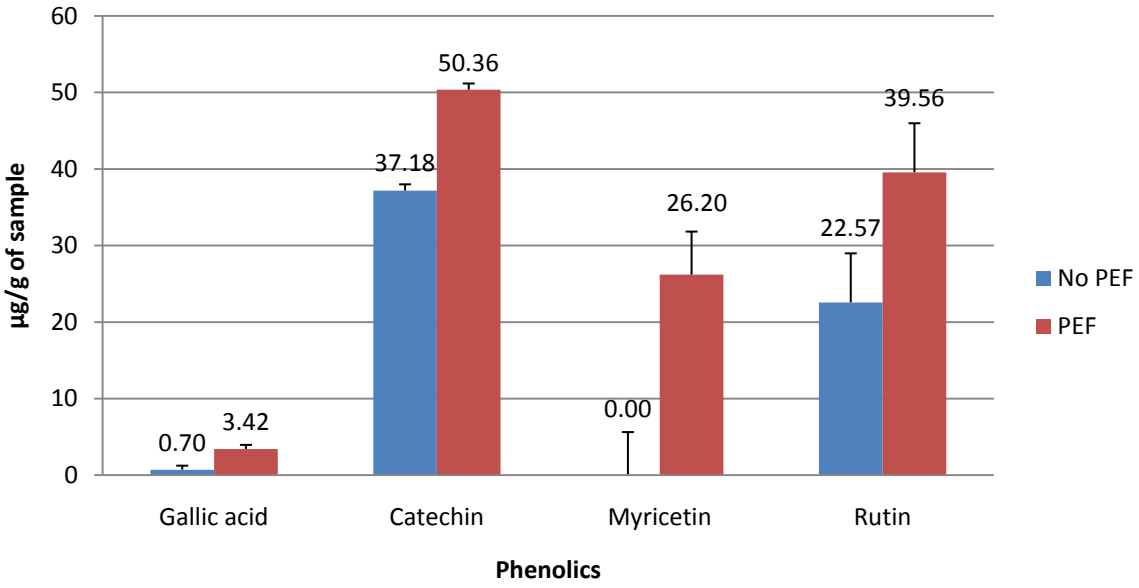


Figure 4.6: Individual phenolics indentified by HPLC in the Flame peel extract subjected to a PEF treatment

Among the identified phenolics, catechin was the most abundant in the extract of the treated samples with an average concentration of 50.36 µg/g of fresh sample. The next abundant was Rutin with 39.56 µg/g followed by myricetin with 26.19 µg/g and lastly by gallic acid with 3.42 µg/g. All these results were significantly different from the control treatments.

Pectinase treated samples

The application of the enzymatic treatment increased the concentration of TPP in the Flame peel extract. Gallic acid, catechin, rutin, myricetin, resveratrol and epicatechin were identified using HPLC. It is interesting to mention that the enzymatic treatment allowed for the extraction of resveratrol and epicatechin. On the other hand, those two phenolics were not identified in the PEF extract. This indicates that depending on the treatment used, the composition of the extract will differ. For all the phenolics identified, the enzymatic treatment was effective in enhancing their concentration compared to the control treatments. Gallic acid concentration increased by 12.9 times, catechin by 1.10 times, rutin by 1.07 times, myricetin by 1.02 times and epicatechin by 1.21 times. The concentration of resveratrol was almost similar between the control and the treated samples. On average, catechin and epicatechin were the most abundant in the treated samples with concentrations of 33.12 and 32.38 µg/g respectively. This is followed by myricetin, resveratrol, rutin and gallic acid with concentrations of 25.92, 15.82, 8.70 and 2.27 µg/g respectively.

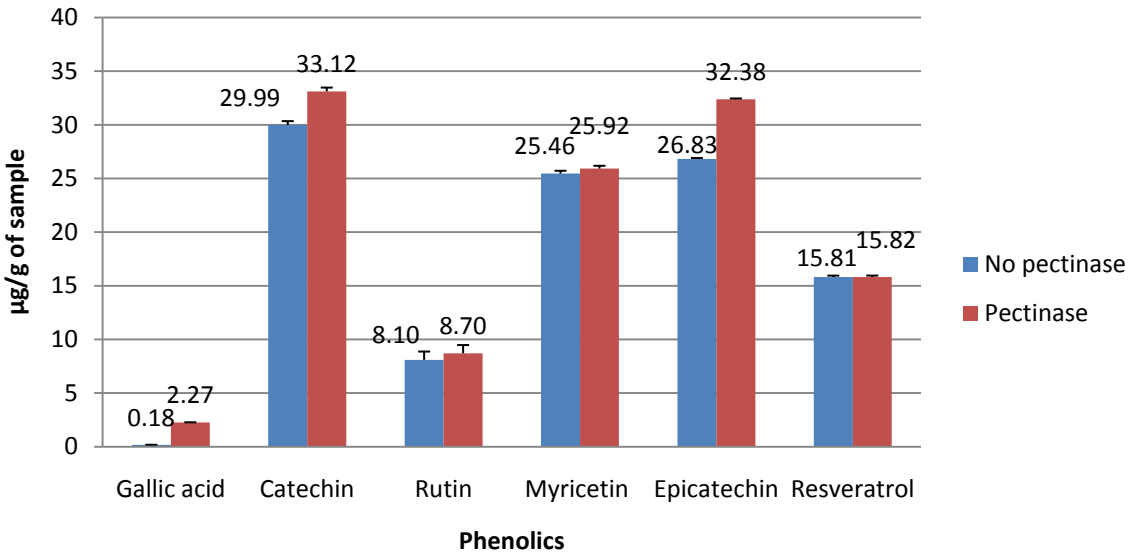


Figure 4.7: Individual phenolics indentified by HPLC in the Flame peel extract subjected to a pectinase treatment

If we compare the effect of both treatments on the concentrations of individual phenolics, we notice that the results match with the total polyphenols results determined earlier. The PEF treatment was more effective than the pectinase treatment in enhancing the extraction of phenolic compounds. In fact, by comparing gallic acid, catechin, rutin and myricetin, we can easily notice that the compounds were more abundant in the PEF extracts. However, in this case, the extract from the pectinase treatment was richer in phenolic compounds where resveratrol and epicatechin were identified. (table 4.1)

Table 4.1: Comparison of the identified phenolics between the PEF and pectinase extracts of the Flame peel

Phenols	**PEF extract (µg/g)**	**Pectinase extract (µg/g)**
Gallic acid	3.42	2.27
Catechin	50.36	33.12
Rutin	26.19	8.70
Myricetin	39.56	25.92
Epicatechin	Not identified	32.38
Resveratrol	Not identified	15.82

Frontenac Pomace

PEF treated samples

The following four phenolics were identified in the extract of the Frontenac pomace: gallic acid, catechin, myricetin and rutin. Compared to the control treatments, the application of the pulsed electric field increased the concentration of gallic acid by 2.67 times, catechin by 1.47 times and rutin by 1.27 times. Myricetin was not identified in the extract of the control treatment. However, about 25.89 µg/g of myricetin were

identified in the extract of the treated pomace. This indicates, that the application of the electrical treatment allows for the extraction of some phenolics that could not be found in the extract of the control treatments.

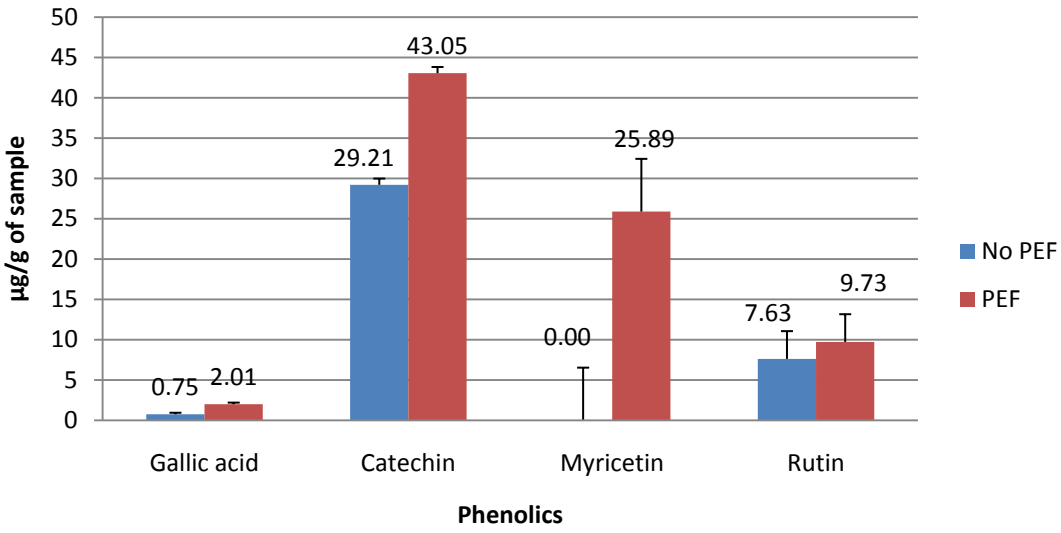


Figure 4.8: Individual phenolics indentified by HPLC in the Frontenac pomace extract subjected to a PEF treatment

Pectinase treated samples

Gallic acid, catechin, myricetin and rutin were identified in the extract of the treated pomace. The application of the pectinase enzymes was effective in increasing the concentration of these individual phenolics compared to the control treatments. The biggest increase was observed for Gallic acid which concentration increased by 1.91 times compared to the control. However, its average concentration remained low (0.1211 µg/g) and not very significant compared to the other identified phenolics. Compared to the control, the application of the enzymatic treatment increased the concentration of epicatechin by 1.38 times and the concentration of catechin by 1.34 times. Myricetin's concentration was not affected very much by the PEF treatment. There was a slight

significant difference between the concentration of myricetin in the control (24.9956 µg/g) and treated samples (25.0389 µg/g).

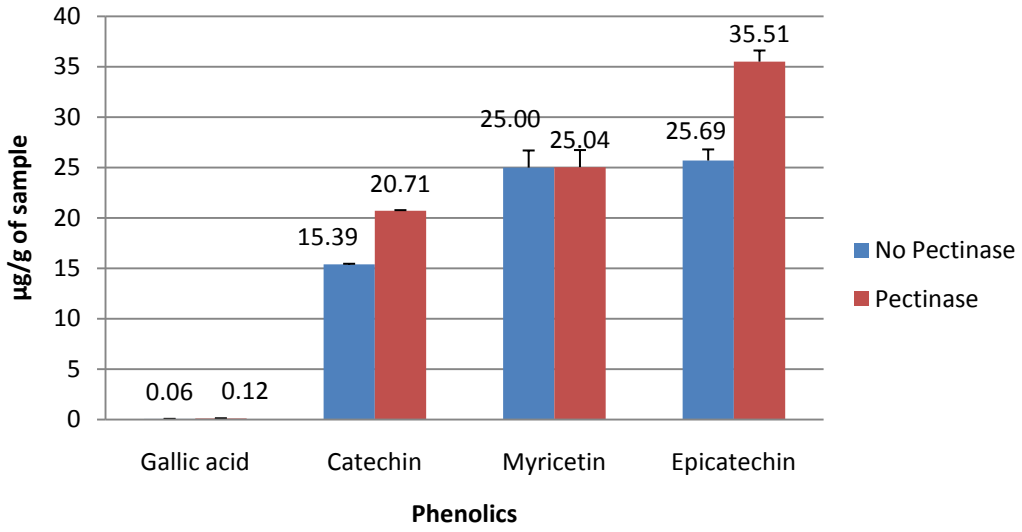


Figure 4.9: Individual phenolics indentified by HPLC in the Frontenac pomace extract subjected to a pectinase treatment

The PEF treatment was more effective in enhancing the extraction of polyphenols from the Frontenac pomace. This was previously confirmed as determined in the total polyphenol concentration. It is important to note that gallic acid, catechin and myricetin were identified in both PEF and pectinase extracts. The extracted myricetin had almost the same concentration between both the PEF and pectinase extract. Rutin which was present in the PEF extract was not identified in the pectinase extract. And, epicatechin which was identified in the pectinase extract was not present in the PEF extract. This proves that the extracts of the same grape cultivar, resulting either from an enzymatic treatment or an electrical treatment might contain different kinds of phenolics.

Table 4.2: Comparison of the identified phenolics between the PEF and pectinase extracts of the Frontenac pomace

Phenols	PEF extract (µg/g)	Pectinase extract (µg/g)
Gallic acid	2.01	0.12
Catechin	43.05	20.71
Myricetin	25.89	25.04
Eicatechin	Not identified	35.51
Rutin	9.73	Not identified

St. Pepin pomace

PEF treated samples

Two phenolic compounds were identified in the pomace of the white grape cultivar St. Pepin. These are gallic acid and catechin. However, there were no significant differences between the control treatments and the treated samples for gallic acid. ($p > 0.05$). In the case of catechin, the PEF treatment increased its concentration in the extract by 1.2 times compared to the control treatment.

Pectinase treated samples

The same two phenolics gallic acid and catechin were identified in the extract of the pomace which was subjected to a pectinase treatment. However, in this case, both compounds did not show any significant differences between the control treatments and the treated samples. Therefore, the application of the enzymatic treatment was not effective in increasing the concentration of these two compounds in the extract.

Table 4.3: Comparison of the identified phenolics between the PEF and pectinase extracts of the St. pepin pomace

Phenols	**PEF extract (µg/g)**	**Pectinase extract (µg/g)**
Gallic acid	0.82	1.08
Catechin	34.30	16.12

St. Croix pomace

PEF treated samples

Gallic acid, catechin, rutin, epicatechin and quercetin were identified in the extract of the St. Croix pomace that has been subjected to a pulsed electric field. The application of the electrical treatment was effective in significantly increasing the concentration of the extracted catechin, epicatechin and quercetin. However, no significant differences were observed for gallic acid and rutin ($p > 0.05$). Compared to the control, the application of the PEF treatment increased the concentration of catechin by 1.63 times, epicatechin by 1.36 times. Quercetin was not identified in the extract of the control treatment. However, an average concentration of 22.23 µg/g was identified in the extract of the treated pomace.

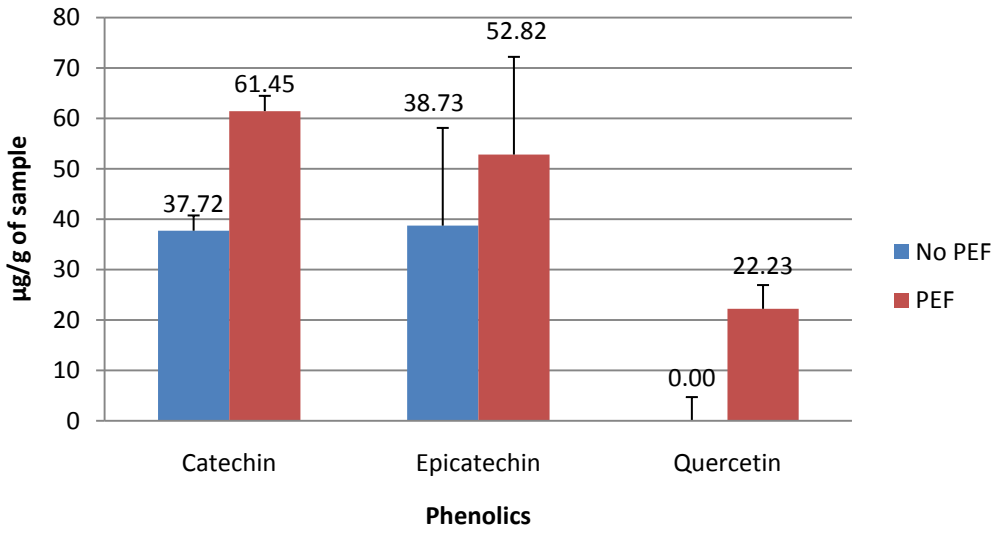


Figure 4.10: Individual phenolics identified in the St. Croix pomace subjected to a PEF treatment

Pectinase treated samples

The following phenolics were identified in the St. Croix pomace subjected to the enzymatic treatment: gallic acid, catechin, epicatechin and quercetin. There were no significant differences between the treated sample and the control for gallic acid. However, the application of the enzymatic treatment significantly increased the concentration of catechin, epicatechin and quercetin. In fact, epicatechin's concentration was higher by 1.77 times than the control. In the case of catechin and quercetin, they were not identified in the extract of the control treatment. However, the extract of the treated samples contained on average 25.96 µg/g of catechin and 22.30 µg/g of quercetin.

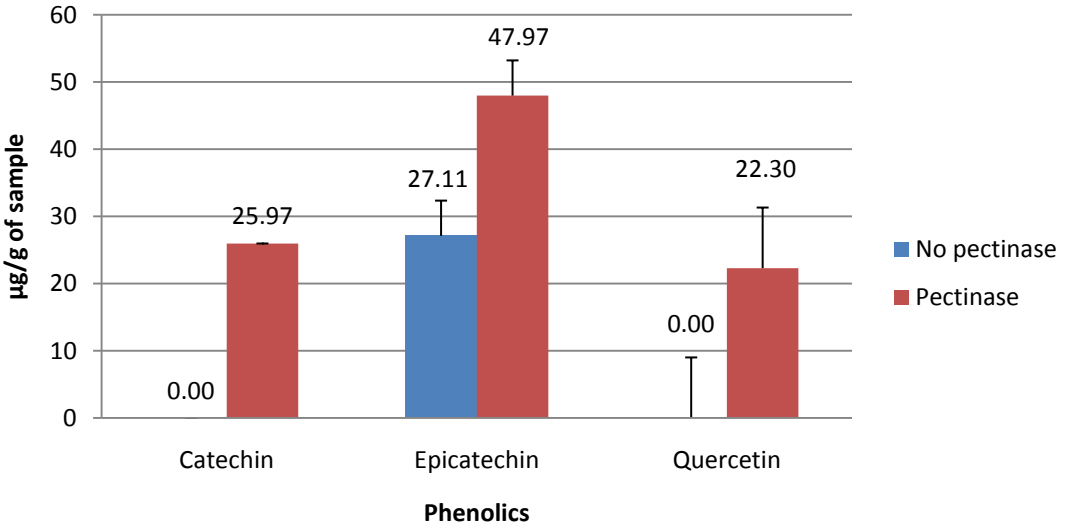


Figure 4.11: Individual phenolics identified in the St. Croix pomace subjected to a pectinase treatment

Almost a similar concentration of quercetin (22 µg/g) was identified in both the PEF and pectinase pomace. For catechin and epicatechin, the PEF treatment was more effective than the pectinase treatement in enhancing the extraction of these 2 phenols. The concentration of catechin was 2.37 times higher in the PEF extract than in the pectinase extract. And the concentration of epicatechin was 1.10 times higherin the PEF extract than in the pectinase extract. Gallic acid was higher in the pectinase extract by 4 times. The PEF treatment was effective in extracting rutin, which was not the case for the pectinase treatment.

Table 4.5: Comparison of the identified phenolics between the PEF and pectinase extracts of the St.Croix pomace

Phenols	PEF extract (µg/g)	Pectinase extract (µg/g)
Gallic acid	0.7179	2.9282
Catechin	61.4464	25.9675
Epicatechin	52.8238	47.9713

Rutin	25.5790	Not identified
Quercetin	22.2250	2.7081

4.3-FRAP: Ferric ion reducing antioxidant power

PEF treated samples

Samples that were exposed to a pulsed electric field showed a higher antioxidant power than those that were not. All results were significantly different. Frontenac showed the highest antioxidant power with an average of 1808.63 µM. On the other hand, St. Pepin had the lowest antioxidant power with an average of 39.92 µM. This is expected for a white grape cultivar. Compared to the control, the application of the pulsed electric field was effective in increasing the antioxidant power by 1.36 times for the Flame peel extract, 1.30 times for St Croix pomace, 1.65 times for Frontenac pomace and 1.14 times for St. Pepin pomace.

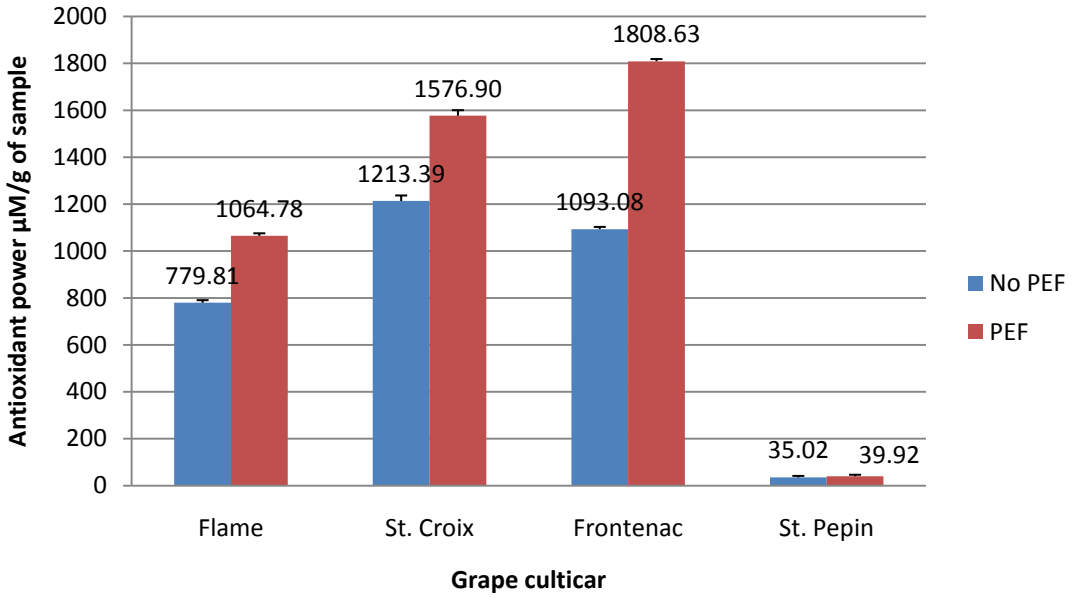


Figure 4.12: Antioxidant power of PEF extracts

Pectinase treated samples

The application of the enzymatic treatment was effective in increasing the antioxidant power of the extracts compared to the control treatments. In this case, St. croix showed the highest antioxidant power with an average of 1345.17 µM in the treated sample. For the pomace of the white cultivar St. Pepin, the antioxidant power was almost close to zero (2.67 µM). The antioxidant power in the extract of the treated flame peel was 2.43 times higher than in the control treatment. Compared to the control, the antioxidant power was 1.58 times higher in the extract of the treated pomace of St. Croix and 1.70 times higher in the extract of the treated pomace of Frontenac.

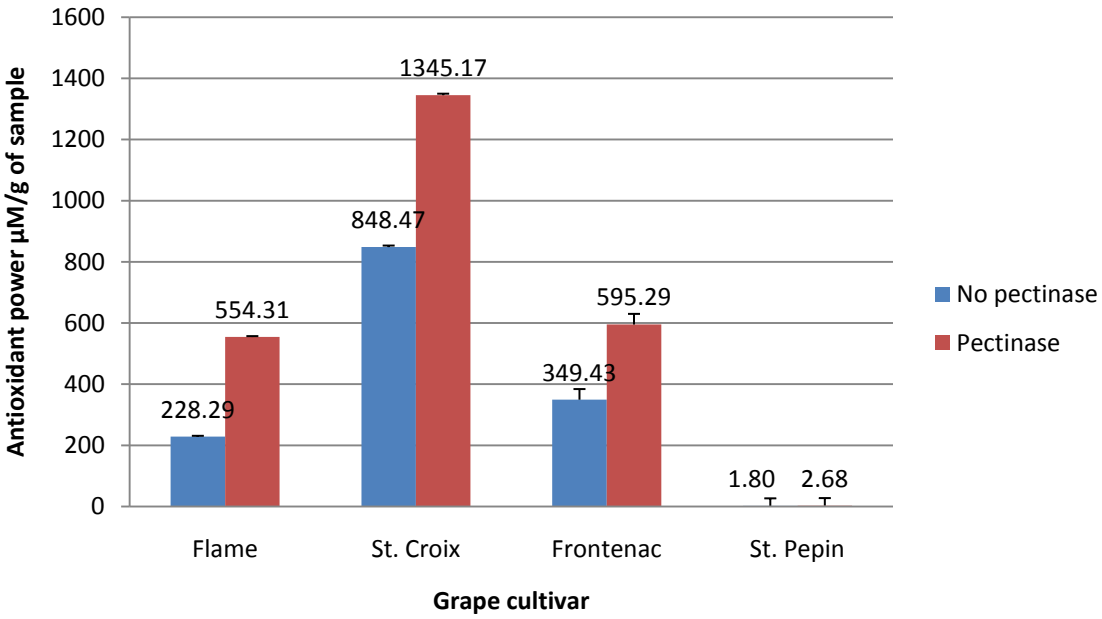


Figure 4.13: Antioxidant power of pectinase extracts

By comparing the antioxidant power in the extracts of both treatments, we notice that the PEF extracts showed a higher antioxidant power than the pectinase extracts. This is expected due to a higher concentration of polyphenols in the PEF extracts. Moreover, it is interesting to mention that for both PEF and pectinase extracts, the antioxidant power

for the Flame cultivar was lower than the St Croix and Frontenac pomace. We saw earlier that the extract from the Flame peel had the highest TPP concentration; therefore we would expect it to have the highest antioxidant power. This was not the case. For that reason, we can conclude that the antioxidant power is not related to the concentration of polyphenols present in the extract. Only the polyphenols, which are characterized by having an antioxidant activity, will determine the antioxidant power.

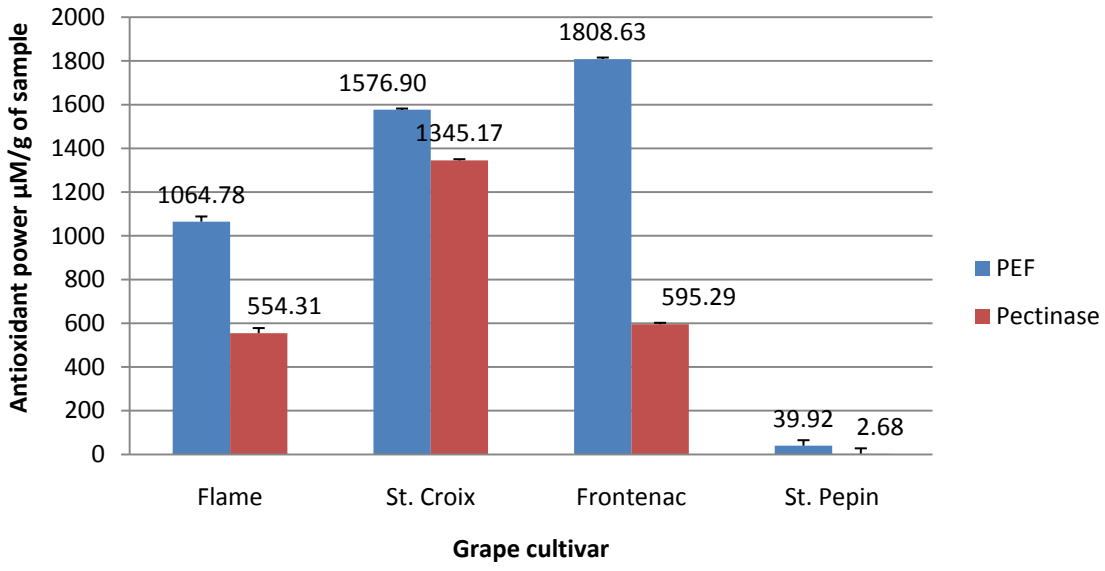


Figure 4.14: Comparison of the antioxidant power between the PEF and pectinase extracts

Chapter 5: Conclusion

The results presented in this work described the effects of the pectinase and pulsed electric field treatments on the extraction of polyphenols from three cultivars of grape pomace and one cultivar of grape peel. In all cases, both treatments were effective in enhancing the extraction of total polyphenols from grape pomace and peel. However, by comparing them together, the application of the PEF showed better results than the pectinase treatment. Therefore, for better extraction efficiency, the use of pulsed electric field is recommended.

The HPLC analysis allowed to identify individual phenolics in the grapes extracts. The concentrention of most identified phenolics, significantly increased by the application of either a PEF or a pectinase treatment. In some cases, extracts of the same grape cultivar which were subjected to either a PEF or a pectinase treatment showed a different composition of phenolics. This is an indication that different phenolics could be extracted depending on the treatment applied. Another interesting phenomenon is that some of the phenolics which could not be identified in the extracts of the control treatments (no PEF or pectinase), were found in the extracts of the treated samples. This indicates that the PEF and pectinase treatments made possible the extraction of some phenols that could not be extracted without the application of either treatment. Therefore, the quality of the extract improved by the application of one of these treatments.

Lastly, the extracts of grape pomace and peel showed a strong antioxidant capacity. This suggests that it can have many potential health benefits. In addition to that, samples that were treated with a PEF or a pectinase treatment exhibited a stronger antioxidant activity than the control treatments. This is explained by the fact that treated extracts are richer in polypehnols and consequently have a stronger antioxidant potential.

Future suggestions

This research is new in its field and will constitute a basis for future studies. For the future, I would suggest the following:

- Analyzing the peel and pomace of all cultivars. In order to do so, grapes should be sampled before they are crushed at the winery. After crushing and pressing, the pomace is sampled too. This will allow a comparison for the phenolics present in both the peel and pomace.
- A cost study on the P.E.F equipment and the operating cost is recommended. This will give a better idea for anyone interested in using this technique at the industrial level. In addition to that, it is suggested to make a cost analysis comparison between the P.E.F and pectinase treatments. This will allow us to make better decisions on which technique would be more efficient to be used at the industrial level.
- It is also suggested to study the importance of the identified phenolics in the extract at the economical level. Since different phenolics were identified depending on the treatment applied, this will help to identify which method (Pulsed electric field or Pectinase) will be more efficient for extracting a particular polyphenol depending on our interest.
- Studying the possibility of applying both treatments (P.E.F and pectinase) together in order to study the effect of a possible positive interaction between both.

References

Balu M., Sangeetha P., Murali G., Panneerselvam C. (2006). Modulatory role of grape seed extract on age-related oxidative DNA damage in central nervous system of rats. Brain Research Bulletin, 68, 469–473

Bamforth W.C. (2005). Food, ferementation and micro-organisms, UK: Blackwell publishing, 216 p.

Bell J.R.C.,Donovan J.L., Wong R., Waterhouse A.L., German J.B., Walzem R.L., Kasim-Karakas S.E. (2000). (+)-Catechin in human plasma after ingestion of a single serving of reconstituted red wine. The American Journal of Clinical Nutrition, 71, 103–108.

Bousetta N., Lebovka N., Vorobiev E., Adenier H., Bedel-Cloutour C., Lanoiselle J.L. (2009). Electrically assisted extraction of soluble matter from chardonnay grape skins for polyphenol recovery. Journal of Agricultural Food Chemistry, 57, 1491-1497

Bravo L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev, 56, 317-333

Breksa P.A., Takeoka R. G., Hidalgo B.M., Vilches A., Vasse J., Ramming W.D. (2010). Antioxidant activity and phenolic content of 16 raisin grape (Vitis vinifera L.) cultivars and selections. Journal of Food Chemistry, 121, 740-745

Brown J.C., Huang G., Haley-Zitlin V., Jiang X.P. (2009). Antibacterial effects of grape extracts on Helicobacter pylori. Applied and Environmental Microbiology, 75, 848–852

Bryan S.N. (2009). Food, nutrition and the nitric oxide pathway: biochemistry and bioactivity. USA: DEStech publications, 218 p.

Chacona M.R., Ceperuelo-Mallafrea V., Maymo-Masipa E., Mateo-Sanzb J.M., Arolac L., Guitierreza C., Fernandez-Reald J.M., Ardevolc A., Simona I., Vendrella. (2009). Grape-seed procyanidins modulate inflammation on human differentiated adipocytes in vitro. Cytokine, 47, 137–142

Cieslik E., Greda A., Adamus W. (2004). Contents of polyphenols in fruit and vegetables. Food Chemistry, 94, 135-142

Creasy L.G., Creasy L.L. (2009). Grapes,UK: CABI, 295 p.

Darlington L. G., Stone T. W. (2001). Antioxidants and fatty acids in the amelioration of rheumatoid arthiritis and related disorders. British Journal of Nutrition, 85, 251–269.

Dell Agli M., Galli G.V., Vrhovsek U., Mattivi F., Bosisio E. (2005). In vitro inhibition of human cGMP-specific phosphodiesterase-5 by polyphenols from red grapes. Journal of Agricultural and Food Chemistry, 53, 1960–1965

Donovan J.L., Bell J.R., Fasim-Karakass S., German J.B., Walzem R.L., Hansen R.J., Waterhouse A.L. (1999). Catechin is present as metabolites in human plasma after consumption of red wine. Journal of Nutrition, 129, 1662-1668

Dunsford P.A., Sneyd T.N. (1989). Pressing for quality. In: Proceedings of the Seventh Australian Wine Industry Technical Conference

FAOSTAT-FAO Statistical database (2010). http://www.fao.org

Flamini R. (2008). Hyphenated techniques in grape and wine chemistry. England: Wiley, 362 p.

God J.M., Tate P., Larcom L.L. (2007). Anticancer effects of four varieties of muscadine grape. Journal of Medicinal Food, 10, 54–59

Gould K., Davies M.K., Winefield C. (2008). Anthocyanins: biosynthesis, functions and applications. (2008). USA: Springer, 329 p.

Halliwell B. (1999) Establishing the significance and optimal intake of dietary antioxidants: The biomarker concept. Nutr Rev, 57, 104-113

Hudson TS, Hartle DK, Hursting SD.(2007). Inhibition of prostate cancer growth by muscadine grape skin extract and resveratrol through distinct mechanisms. Cancer Res. 67 (17), 8396–405

Jackson S.R. (2008). Wine science: principles and applications, USA: academic press, third edition, 475 p.

Kammerer D., Claus A., Carle R., Schieber A. (2004). Polyphenol screening of pomace from red and white grape varieties (Vitis vinifera L.) by HPLC-DAD-MS/MS. Journal of Agricultural and Food Chemistry, 52, 4360-4367

Lanzarini G., Pifferi P.G. (1989). Enzymes in the fruit juice industry. Biotechnology applications in beverage production. London: Elsevier Science Publishers Ltd, 189-222

Lazze M.C., Pizzala R., Pecharroman F.J.G., Garnica P.G., Rodriguez J.M.A., Fabris N., Bianchi L. (2009). Grape waste extract obtained by supercritical fluid extraction contains bioactive antioxidant molecules and induces antiproliferative effects in human colon adenocarcinoma cells. Journal of Medicinal Food, 12, 561–568

Lean M.E., Noroozi M., Kelly I., Burns J., Talwar D., Sattar N., Crozier A. (1999). Dietary flavonols protect diabetic human lymphocytes against oxidative damage to DNA. Diabetes, 48,176-181

Makris P. D., Boskou G. (2008). Characterisation of certain major polyphenolic antioxidants in grape (Vitis vinifera cv. Rotidis) stems by liquid chromatography-mass spectrometry. European Food Research and Technology, 226, 1075-1079

Manach C., Scalbert A., Morand C., Remesy C., Jimenez L. (2004). Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition, 79, 727-747

Masoro J.E., Austad N.S. (2006). Handbook of the biology of aging. UK: Academic press, sixth edition, 680 p.

Mcanlis G.T., Mceneny J., Pearce J., Young I.S. (1999). Absorption and antioxidant effects of quercetin from onions, in man. European Journal of Clinical Nutrition, 53,92-96

Meyer S.A., Jepsen M.S., Sorensen S.N. (1998). Enzymatic release of antioxidants for human low-density lipoprotein from grape pomace. Journal of Agricultural and Food Chemistry, 46 (7), 2339-2446

Morand C., Crespy V., Manach C., Besson C., Demigne C., Remesy C. (1998). Plasma metabolites of quercetin and their antioxidant properties. American Journal of Physiology, 275, R212-R219

Negro C., Tommasi L., Miceli A. (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresource Technology, 87, 41-44

Nigdikar S.V., Williams N.R., Griffin B.A., Howard A.N. (1998). Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation in vivo. The American Journal of Clinical Nutrition, 68, 258-265

OIV, office international de la vigne et du vin (2006). Paris, France

Okushio K., Suzuki M., Matsumoton N., Nanjo F., Hara Y. (1999a). Identification of (-)-epicatechin metabolites and their metabolic fate in the rat. Drug Metabolism and Disposition, 27,309-316

Okushio K., Suzuki M., Matsumoton N., Nanjo F., Hara Y. (1999b). Methylation of tea catechins by rat liver homogenates. Bioscience, Biotechnolgy and Biochemistry, 63,430-432

Palmer T., Bonner L.P. (2007). Enzymes : biochemistry, biotechnology, clinical chemistry. England: Woodhead publishing, second edition, 432 p.

Papadopoulou C., Soulti K., Roussis I.G. (2005). Potential antimicrobial activity of red and white wine phenolic extracts against strains of Taphylococcus aureus, Escherichia coli and Candida albicans. Food Technology and Biotechnology, 43, 41–46

Pietta P.G., Simonetti P., Gardana C., Rusamolino A., Morazzoni P., Bombardelli E. (1998). Catechin metabolites after intake of green tea infusions. Biofactors, 8,111-118

Piksula M.K.,Terao J. (1998). Accumulation of (-) epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. Journal of Nutrition, 128, 1172-1178

Pinelo M., Arnous A., Meyer S.A. (2006). Upgrading of grape skins: significance of plant cell-wall structural components and extraction techniques for phenol release. Trends in Food Science and Technology, 17, 579-590

Qiu X., Sharma S., Tuhela L., Jia M., Zhang Q. H. (1998). An integrated PEF pilot plant for continuous nonthermal pasteurization of fresh orange juice. Transactions of ASAE, 41 (4), 1069-1074

Rose K.C.J. (2003). The plant cell wall. UK: Blackwell publishing, 381p.

Scalbert A., Johnson T.I., Saltmarsh M. (2005). Polyphenols: antioxidants and beyond. American Journal of Clinical Nutrition, 81, 215S-217S

Scalbert A., Williamson G. (2000). Dietary intake and bioavailability of Polyphenols. The Journal of Nutrition, 130, 2073S-2085S

Shetty K., Paliyath G., Pometto L.A. (2006). Functional foods and biotechnology. Florida: CRC Press, 653 p.

Sivarooban T., Hettiarachchy N.S., Johnson M.G. (2008). Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. Food Research International, 41, 781–785

Soobrattee M.A., Neergheena V.S., Luximon-Rammaa A., Aruomab O.I., Bahoruna T. (2005). Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutation Research Fundamental and Molecular Mutagen, 579, 200–213

Suarez B., Santamaria J., Mangas J.J., Blanco D. (1994). High-Performance liquid chromatography of the neutral phenolic compounds of low molecular weight in apple juice. Journal of Agicultural and Food Chemistry, 42, 2732

Terra X., Montagut G, Bustos M., Llopiz N., Ardevol A., Blade C., Fernandez-Larrea J., Pujadas G., Salvado, J., Arola L. (2009). Grape-seed procyanidins prevent low-grade inflammation by modulating cytokine expression in rats fed a high-fat diet. The Journal of Nutritional Biochemistry, 20, 210–218

Thimothe J., Bonsi I.A., Padilla-Zakour O.I. (2007). Chemical characterization of red wine grape (Vitis vinifera and Vitis Interspecific Hybrids) and pomace phenolic extracts and their biological activity against Streptococcus mutans. Journal of Agricultural Food Chemistry, 55, 10200–10207

Whitaker R.J., (1984). Pectic substances, pectic enzymes and haze formation in fruit juices. Enzyme Microb. Technol. 6:341-349

Whitaker R.J. (1994). Principles of enzymology for the food sciences. USA: Marcel Dekker, second edition, 625 p.Whitehurst J.R., Van Oort M. (2009). Enzymes in food technology, UK: Wiley-Blackwell, second edition, 384 p.

Xia E.Q., Deng G.F., Guo Y.J., Li H.B. (2010). Biological activities of polyphenols from grapes. International journal of molecular sciences, 11, 622-646

Yilmaz Y., Toledo T.R. (2004). Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. Journal of Agricultural and Food chemistry, 52, 255-260

Appendices

Appendix A. SAS codes used for statistical analysis of response variables during the preliminary study

Impedance

```
data impedance;
input pulse @;
do obs= 1 to 3;
input imp @;
output;
end;
datalines;

  0  163.4  171.78 156.5
  5  97.2   108.20 111.08
  10 85.13  90.81  86.43
  15 79.62  85.21  81.57
  20 75.54  77.44  72.26
  25 72.48  74.55  67.48
  30 70.44  73.28  65.08
  35 69.05  71.36  63.50
  40 67.75  70.08  62.52
  45 66.26  67.87  61.67
  50 65.33  67.19  60.66
  55 64.60  66.73  60.50
  60 63.99  65.40  59.18
  ;

proc print data=impedance;
run;
proc mixed data=impedance;
class pulse;
model imp=pulse;
lsmeans pulse;
estimate '0 vs 5' pulse 1 -1 0 0 0 0 0 0 0 0 0 0 0;
estimate '5 vs 10' pulse 0 1 -1 0 0 0 0 0 0 0 0 0 ;
estimate '10 vs 15' pulse 0 0 1 -1 0 0 0 0 0 0 0 0 0;
estimate '15 vs 20' pulse 0 0 0 1 -1 0 0 0 0 0 0 0 0;
estimate '20 vs 25' pulse 0 0 0 0 1 -1 0 0 0 0 0 0 0;
run;
```

Duration of water bath

```
data waterbath;
input type $ duration @;
do obs= 1 to 3;
input tpp @;
output;
end;
datalines;

 C 30 2527.6732 2574.1433 2519.3190
 T 30 2918.7531 2850.3533 2859.2296
 C 60 3603.7957 3671.4123 3670.6291
 T 60 4644.4129 4732.9150 4633.7091
 C 90 3699.6077 3676.1115 3707.4397
 T 90 4654.3335 4627.9656 4730.3043
 ;


 proc mixed data=waterbath;
 class type duration;
 model tpp= type duration type*duration;
 lsmeans type*duration / diff;
 run;
```

Temperature of water bath

```
data temperature;
input type $ temp @;
do obs= 1 to 3;
input tpp @;
output;
end;
datalines;

  C 30 2533.6778 2540.4655 2598.9448
  T 30 4203.4690 3784.4548 3920.9934
  C 40 5616.8914 5999.3560 5500.7161
  T 40 6351.7979 6228.5738 6418.8924
  C 50 6442.3885 6636.3621 6897.9522
  T 50 8092.8607 8048.7402 8150.2956
  C 60 6351.0147 6316.8148 6481.8098
  T 60 7576.9902 7465.5142 7828.6598
  C 70 6308.7217 6032.2506 6427.2466
  T 70 7193.4813 7014.1275 7260.3147
  ;

proc mixed data=temperature;
class type temp;
model tpp= type temp type*temp;
lsmeans type*temp / diff;
run;
```

Duration of pectinase incubation

```
data pectinase;
input type $ duration @;
do obs= 1 to 3;
input tpp @;
output;
end;
datalines;

C 1 2437.083 2522.191 2485.641
T 1 3247.96  3145.099 3004.122
C 2 2548.037 2489.557 2514.62
T 2 3458.12  3493.103 3469.868
C 3 2537.594 2565.006 2527.673
T 3 3418.176 3424.964 3420.004
;

proc mixed data=pectinase;
class type duration;
model tpp= type duration type*duration;
lsmeans type*duration / diff;
run;
```

Appendix B. SAS codes used for statistical analysis of response variables during PEF and pectinase treatments of grape pomace and peel

Total polyphenols PEF

```
data PEF;
input  variety $ block $ @@;
do trt= 1 to 2;
input tpp @;
output;
end;
datalines;

flame     a 6580.5805 8097.2988
flame     b 6501.1288 8101.0408
flame     c 6551.8630 8109.2209
frontenac a 2295.9318 2896.2143
frontenac b 2313.4234 2842.7823
frontenac c 2287.2295 2858.3594
stcroix   a 3648.6124 4670.3457
stcroix   b 3642.9559 4646.8495
stcroix   c 3629.2063 4670.2863
stpepin   a 418.8327  466.4428
stpepin   b 418.1974  467.5306
stpepin   c 418.7631  467.2173
;

proc print;
run;
proc mixed data=PEF;
class variety block trt;
model tpp= trt block variety trt*variety block*trt;
lsmeans trt*variety/diff;
run;
```

Total polyphenols pectinase

```
data PEC;
input  variety $ block $ @@;
do trt= 1 to 2;
input tpp @;
output;
end;
datalines;

flame     a 2925.1928 3361.3502
flame     b 2868.8021 3387.2833
flame     c 2901.5226 3385.4558
frontenac a 1197.7926 1810.2582
frontenac b 1234.6902 1820.7880
frontenac c 1206.1468 1812.5208
stcroix   a 2878.1135 3193.5708
```

```
stcroix   b 2894.5608 3204.3616
stcroix   c 2880.5502 3199.0532
stpepin   a 159.8184  201.3021
stpepin   b 146.6345  202.3638
stpepin   c 147.1740  205.6968
;

proc print;
run;
proc mixed data=PEC;
class variety block trt;
model tpp= trt block variety trt*variety block*trt;
lsmeans trt*variety/diff;
run;
```

HPLC – Frontenac, PEF

```
data frontenacPEF;
input grape $ phenoltrt $ @;
do rep = 1 to 3;
input conc @;
output;
end;
datalines;

Frontenac GAc 0.5848 0.9672 0.7040
Frontenac GAp 1.5936 2.9005 1.5295
Frontenac Catc 36.7560 25.8122 25.0757
Frontenac Catp 41.1419 40.9835 47.0193
Frontenac Rutc 7.5458 6.9834 8.3552
Frontenac Rutp 10.1651 7.0020 12.0108
Frontenac Myrc 0       0       0
Frontenac Myrp 25.5357 25.7810 26.3514
;

proc print data= frontenacPEF;
run;
proc mixed data=frontenacPEF;
class phenoltrt;
model conc=phenoltrt;
lsmeans phenoltrt;
estimate 'GAc vs GAp' phenoltrt 0 0 1 -1 0 0 0 0;
estimate 'Catc vs Catp' phenoltrt 1 -1 0 0 0 0 0 0;
estimate 'Rutc vs Rutp' phenoltrt 0 0 0 0 0 0 1 -1;
estimate 'Myrc vs Myrp' phenoltrt 0 0 0 0 1 -1 0 0;
run;
```

HPLC – Fronteneac, pectinase

```
data frontenacPEC;
input grape $ phenoltrt $ @;
do rep = 1 to 3;
input conc @;
output;
end;
datalines;

frontenac GAc 0.0641 0.0686 0.0574
fronteanc GApec 0.1664 0.0551 0.1417
frontenac Catc 17.3433 14.2904 14.5467
frontenac Catpec 19.8462 21.9529 20.3402
frontenac Myrc 24.9936 24.9801 25.0132
frontenac Myrpec 25.0573 25.0242 25.0353
frontenac Epicatc 31.9446 20.1626 24.9706
frontenac Epicatpec 35.8167 34.9182 35.7846
;

proc print data= frontenacPEC;
run;
proc mixed data=frontenacPEC;
class phenoltrt;
model conc=phenoltrt;
lsmeans phenoltrt;
estimate 'GAc vs GApec' phenoltrt 0 0 0 0 1 -1 0 0 ;
estimate 'Catc vs Catpec' phenoltrt 1 -1 0 0 0 0 0 0 ;
estimate 'Myrc vs Myrpec' phenoltrt 0 0 0 0 0 0 1 -1 ;
estimate 'Epicatc vs Epicatpec' phenoltrt 0 0 1 -1 0 0 ;
run;
```

HPLC – St.Croix, PEF

```
data stcroixPEF;
input grape $ phenoltrt $ @;
do rep = 1 to 3;
input conc @;
output;
end;
datalines;

stcroix GAc 0.0169 0.0866 0.1282
stcroix GAp 1.1123 0.8604 0.1811
stcroix Catc 35.4509 36.5463 41.1606
stcroix Catp 74.4768 39.1657 70.6968
stcroix Rutc 14.4515 27.5949 26.6907
stcroix Rutp 19.7601 22.3189 18.9025
stcroix Epicatc 37.0467 35.0840 44.0475
stcroix Epicatp 54.8294 51.8558 51.7863
stcroix Querc   0       0       0
stcroix Querp 22.2126 22.3410 22.1214
;
proc print data= stcroixPEF;
run;
```

```
proc mixed data=stcroixPEF;
class phenoltrt;
model conc=phenoltrt;
lsmeans phenoltrt;
estimate 'GAc vs GAp' phenoltrt 0 0 0 0 1 -1 0 0 0 0;
estimate 'Catc vs Catp' phenoltrt 1 -1 0 0 0 0 0 0 0 0;
estimate 'Rutc vs Rutp' phenoltrt 0 0 0 0 0 0 0 0 1 -1;
estimate 'Epicatc vs Epicatp' phenoltrt 0 0 1 -1 0 0 0 0 0 0;
estimate 'Querc vs Querp' phenoltrt 0 0 0 0 0 0 1 -1 0 0;
run;
```

HPLC – St.Croix, pectinase

```
data stcroixPEC;
input grape $ phenoltrt $ @;
do rep = 1 to 3;
input conc @;
output;
end;
datalines;

stcroix GAc 0.0574 0.0360 0.0664
stcroix GApec 6.5151 0.0742 2.1953
stcroix Catc 0        0      0
stcroix Catpec 31.7968 21.6406 24.4652
stcroix Epicatc 35.1000 17.3334 28.8854
stcroix Epicatpec 56.5408 42.5072 44.8658
stcroix Querc 0          0        0
st croix Querpec 22.2198 22.3652 22.3189
;

proc print data= stcroixPEC;
run;
proc mixed data=stcroixPEC;
class phenoltrt;
model conc=phenoltrt;
lsmeans phenoltrt;
estimate 'GAc vs GApec' phenoltrt 0 0 0 0 1 -1 0 0;
estimate 'Catc vs Catpec' phenoltrt 1 -1 0 0 0 0 0 0;
estimate 'Querc vs Quercpec' phenoltrt 0 0 0 0 0 0 1 -1;
estimate 'Epicatc vs Epicatpec' phenoltrt 0 0 1 -1 0 0;
run;
```

HPLC – St.Pepin, PEF

```
data stpepinPEF;
input grape $ phenoltrt $ @;
do rep = 1 to 3;
input conc @;
output;
end;
datalines;

stpepin GAc 0.3340 0.5994 0.3138
stpepin GAp 0.6033 1.2513 0.6114
```

```
stpepin Catc 29.3144 25.6695 30.4610
stpepin Catp 34.3016 33.9100 34.6977
;

proc print data= stpepinPEF;
run;
proc mixed data=stpepinPEF;
class phenoltrt;
model conc=phenoltrt;
lsmeans phenoltrt;
estimate 'GAc vs GAp' phenoltrt 0 0 1 -1 ;
estimate 'Catc vs Catp' phenoltrt 1 -1 0 0 ;
run;
```

HPLC – St.Pepin, pectinase

```
data stpepinPEC;
input grape $ phenoltrt $ @;
do rep = 1 to 3;
input conc @;
output;
end;
datalines;

stpepin GAc  0       0       0
stpepin GApec 1.1100 1.0489 1.0833
stpepin Catc 19.1909 5.7767 8.5267
stpepin Catpec 7.1517 15.9888 25.2174
;

proc print data= stpepinPEC;
run;
proc mixed data=stpepinPEC;
class phenoltrt;
model conc=phenoltrt;
lsmeans phenoltrt;
estimate 'GAc vs GApec' phenoltrt 0 0 1 -1;
estimate 'Catc vs Catpec' phenoltrt 1 -1 0 0;
run;
```

HPLC – Flame, PEF

```
data flamePEF;
input grape $ phenoltrt $ @;
do rep = 1 to 3;
input conc @;
output;
end;
datalines;

Flame  GAc   1.1100 0.8964 0.1046
Flame  GAp   3.8947 2.4877 3.8722
Flame  Catc  30.8460 41.5661 39.1377
Flame  Catp 55.2365 43.1042 52.7476
```

```
Flame  Rutc  24.8963 30.6725 12.1273
Flame  Rutp  32.2432 49.7895 36.6616
Flame  Myrc  0       0       0
Flame  Myrp  25.7443 27.0849 25.7602
;

proc print data= phenolics;
run;
proc mixed data=phenolics;
class phenoltrt;
model conc=phenoltrt;
lsmeans phenoltrt;
estimate 'GAc vs GAp' phenoltrt 0 0 1 -1 0 0 0 0;
estimate 'Catc vs Catp' phenoltrt 1 -1 0 0 0 0 0 0;
estimate 'Rutc vs Rutp' phenoltrt 0 0 0 0 0 0 1 -1;
estimate 'Myrc vs Myrp' phenoltrt 0 0 0 0 1 -1 0 0;
run;
```

HPLC – Flame, pectinase

```
data flamePEC;
input grape $ phenoltrt $ @;
do rep = 1 to 3;
input conc @;
output;
end;
datalines;

flame GAc 0.1732 0.1833 0.1709
flame GApec 2.6396 1.9378 2.2302
flame Catc 30.7015 29.1634 30.1049
flame Catpec 32.9807 32.9574 33.4141
flame Rutc 8.1455 8.0057 8.1362
flame Rutpec 8.6038 8.8524 8.6411
flame Myrc 25.3591 25.4069 25.6167
flame Myrpec 25.8436 26.0055 25.9184
flame Resc 15.8133 15.8166 15.8085
flame Respec 15.8089 15.8126 15.8277
flame Epicatc 27.4628 26.6980 26.3183
flame Epicatpec 32.6559 32.2601 32.2120
;

proc print data= flamePEC;
run;
proc mixed data=flamePEC;
class phenoltrt;
model conc=phenoltrt;
lsmeans phenoltrt;
estimate 'GAc vs GApec' phenoltrt 0 0 0 0 1 -1 0 0 0 0 0 0;
estimate 'Catc vs Catpec' phenoltrt 1 -1 0 0 0 0 0 0 0 0 0 0;
estimate 'Rutc vs Rutpec' phenoltrt 0 0 0 0 0 0 0 0 0 0 1 -1;
estimate 'Myrc vs Myrpec' phenoltrt 0 0 0 0 0 0 1 -1 0 0 0 0;
estimate 'Resc vs Respec' phenoltrt 0 0 0 0 0 0 0 0 1 -1 0 0;
estimate 'Epicatc vs Epicatpec' phenoltrt 0 0 1 -1 0 0 0 0 0 0;
run;
```

FRAP - PEF

```
data PEFfrap;
input  variety $ block $ @@;
do trt= 1 to 2;
input tpp @;
output;
end;
datalines;

flame     a 782.6667  1091.5833
flame     b 788.9167  1047.3333
flame     c 767.8333  1055.4167
frontenac a 1101.1875 1808.9375
frontenac b 1086.4375 1789.9375
frontenac c 1091.6250 1827.0000
stcroix   a 1207.6250 1581.5833
stcroix   b 1224.7917 1579.7917
stcroix   c 1207.7500 1569.3333
stpepin   a 36.0042   40.1000
stpepin   b 34.0875   39.7875
stpepin   c 34.9625   39.8792
;

proc print;
run;
proc mixed data=PEFfrap;
class variety block trt;
model tpp= trt block variety trt*variety block*trt;
lsmeans trt*variety/diff;
run;
```

FRAP - pectinase

```
data PECfrap;
input  variety $ block $ @@;
do trt= 1 to 2;
input tpp @;
output;
end;
datalines;

flame     a 225.6667 557.0417
flame     b 227.5417 557.4583
flame     c 231.6667 548.4167
frontenac a 346.4583 605.4167
frontenac b 359.3333 584.7917
frontenac c 342.5000 595.6667
stcroix   a 812.3333 1373.6667
stcroix   b 851.2500 1335.0833
stcroix   c 881.8333 1326.7500
stpepin   a 1.7250   2.8417
stpepin   b 1.8963   2.5833
stpepin   c 1.7913   2.6000
;
```

```
proc print;
run;
proc mixed data=PECfrap;
class variety block trt;
model tpp= trt block variety trt*variety block*trt;
lsmeans trt*variety/diff;
run;
```

Appendix C. SAS output for statistical analysis of response variables during the preliminary study

Table 5.1: Estimates and LS means for the impedance measurements during the preliminary study

Estimates					
Label	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
0 vs 5	58.4000	3.5095	26	16.64	<.0001
5 vs 10	18.0367	3.5095	26	5.14	<.0001
10 vs 15	5.3233	3.5095	26	1.52	0.1414
15 vs 20	7.0533	3.5095	26	2.01	0.0549
20 vs 25	3.5767	3.5095	26	1.02	0.3175

Least Squares Means						
Effect	**pulse**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
pulse	0	163.89	2.4816	26	66.04	<.0001
pulse	5	105.49	2.4816	26	42.51	<.0001
pulse	10	87.4567	2.4816	26	35.24	<.0001
pulse	15	82.1333	2.4816	26	33.10	<.0001
pulse	20	75.0800	2.4816	26	30.25	<.0001
pulse	25	71.5033	2.4816	26	28.81	<.0001
pulse	30	69.6000	2.4816	26	28.05	<.0001
pulse	35	67.9700	2.4816	26	27.39	<.0001
pulse	40	66.7833	2.4816	26	26.91	<.0001
pulse	45	65.2667	2.4816	26	26.30	<.0001
pulse	50	64.3933	2.4816	26	25.95	<.0001
pulse	55	63.9433	2.4816	26	25.77	<.0001
pulse	60	62.8567	2.4816	26	25.33	<.0001

Table 5.2: LS means for the "Duration of water bath" preliminary study

Least Squares Means							
Effect	**type**	**duration**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
type*duration	C	30	2540.38	23.3529	12	108.78	<.0001
type*duration	C	60	3648.61	23.3529	12	156.24	<.0001
type*duration	C	90	3694.39	23.3529	12	158.20	<.0001
type*duration	T	30	2876.11	23.3529	12	123.16	<.0001
type*duration	T	60	4670.35	23.3529	12	199.99	<.0001
type*duration	T	90	4670.87	23.3529	12	200.01	<.0001

Differences of Least Squares Means									
Effect	**type**	**duration**	**_type**	**_duration**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
type*duration	C	30	C	60	-1108.23	33.0260	12	-33.56	<.0001

Differences of Least Squares Means									
Effect	type	duration	_type	_duration	Estimate	Standard Error	DF	t Value	Pr > |t|
type*duration	C	30	C	90	-1154.01	33.0260	12	-34.94	<.0001
type*duration	C	30	T	30	-335.73	33.0260	12	-10.17	<.0001
type*duration	C	30	T	60	-2129.97	33.0260	12	-64.49	<.0001
type*duration	C	30	T	90	-2130.49	33.0260	12	-64.51	<.0001
type*duration	C	60	C	90	-45.7739	33.0260	12	-1.39	0.1910
type*duration	C	60	T	30	772.50	33.0260	12	23.39	<.0001
type*duration	C	60	T	60	-1021.73	33.0260	12	-30.94	<.0001
type*duration	C	60	T	90	-1022.26	33.0260	12	-30.95	<.0001
type*duration	C	90	T	30	818.27	33.0260	12	24.78	<.0001
type*duration	C	90	T	60	-975.96	33.0260	12	-29.55	<.0001
type*duration	C	90	T	90	-976.48	33.0260	12	-29.57	<.0001
type*duration	T	30	T	60	-1794.23	33.0260	12	-54.33	<.0001
type*duration	T	30	T	90	-1794.76	33.0260	12	-54.34	<.0001
type*duration	T	60	T	90	-0.5221	33.0260	12	-0.02	0.9876

Table 5.3: LS means for the “Temperature of water bath” preliminary study

Least Squares Means							
Effect	type	temp	Estimate	Standard Error	DF	t Value	Pr > |t|
type*temp	C	30	2557.70	96.3817	20	26.54	<.0001
type*temp	C	40	5705.65	96.3817	20	59.20	<.0001
type*temp	C	50	6658.90	96.3817	20	69.09	<.0001
type*temp	C	60	6383.21	96.3817	20	66.23	<.0001
type*temp	C	70	6256.07	96.3817	20	64.91	<.0001
type*temp	T	30	3969.64	96.3817	20	41.19	<.0001
type*temp	T	40	6333.09	96.3817	20	65.71	<.0001
type*temp	T	50	8097.30	96.3817	20	84.01	<.0001
type*temp	T	60	7623.72	96.3817	20	79.10	<.0001
type*temp	T	70	7155.97	96.3817	20	74.25	<.0001

Differences of Least Squares Means									
Effect	type	temp	_type	_temp	Estimate	Standard Error	DF	t Value	Pr > |t|
type*temp	C	30	C	40	-3147.96	136.30	20	-23.10	<.0001
type*temp	C	30	C	50	-4101.20	136.30	20	-30.09	<.0001
type*temp	C	30	C	60	-3825.52	136.30	20	-28.07	<.0001
type*temp	C	30	C	70	-3698.38	136.30	20	-27.13	<.0001
type*temp	C	30	T	30	-1411.94	136.30	20	-10.36	<.0001
type*temp	C	30	T	40	-3775.39	136.30	20	-27.70	<.0001
type*temp	C	30	T	50	-5539.60	136.30	20	-40.64	<.0001
type*temp	C	30	T	60	-5066.03	136.30	20	-37.17	<.0001
type*temp	C	30	T	70	-4598.28	136.30	20	-33.74	<.0001
type*temp	C	40	C	50	-953.25	136.30	20	-6.99	<.0001
type*temp	C	40	C	60	-677.56	136.30	20	-4.97	<.0001

Differences of Least Squares Means									
Effect	type	temp	_type	_temp	Estimate	Standard Error	DF	t Value	Pr > |t|
type*temp	C	40	C	70	-550.42	136.30	20	-4.04	0.0006
type*temp	C	40	T	30	1736.02	136.30	20	12.74	<.0001
type*temp	C	40	T	40	-627.43	136.30	20	-4.60	0.0002
type*temp	C	40	T	50	-2391.64	136.30	20	-17.55	<.0001
type*temp	C	40	T	60	-1918.07	136.30	20	-14.07	<.0001
type*temp	C	40	T	70	-1450.32	136.30	20	-10.64	<.0001
type*temp	C	50	C	60	275.69	136.30	20	2.02	0.0567
type*temp	C	50	C	70	402.83	136.30	20	2.96	0.0078
type*temp	C	50	T	30	2689.26	136.30	20	19.73	<.0001
type*temp	C	50	T	40	325.81	136.30	20	2.39	0.0268
type*temp	C	50	T	50	-1438.40	136.30	20	-10.55	<.0001
type*temp	C	50	T	60	-964.82	136.30	20	-7.08	<.0001
type*temp	C	50	T	70	-497.07	136.30	20	-3.65	0.0016
type*temp	C	60	C	70	127.14	136.30	20	0.93	0.3621
type*temp	C	60	T	30	2413.57	136.30	20	17.71	<.0001
type*temp	C	60	T	40	50.1251	136.30	20	0.37	0.7169
type*temp	C	60	T	50	-1714.09	136.30	20	-12.58	<.0001
type*temp	C	60	T	60	-1240.51	136.30	20	-9.10	<.0001
type*temp	C	60	T	70	-772.76	136.30	20	-5.67	<.0001
type*temp	C	70	T	30	2286.43	136.30	20	16.77	<.0001
type*temp	C	70	T	40	-77.0151	136.30	20	-0.57	0.5783
type*temp	C	70	T	50	-1841.23	136.30	20	-13.51	<.0001
type*temp	C	70	T	60	-1367.65	136.30	20	-10.03	<.0001
type*temp	C	70	T	70	-899.90	136.30	20	-6.60	<.0001
type*temp	T	30	T	40	-2363.45	136.30	20	-17.34	<.0001
type*temp	T	30	T	50	-4127.66	136.30	20	-30.28	<.0001
type*temp	T	30	T	60	-3654.08	136.30	20	-26.81	<.0001
type*temp	T	30	T	70	-3186.34	136.30	20	-23.38	<.0001
type*temp	T	40	T	50	-1764.21	136.30	20	-12.94	<.0001
type*temp	T	40	T	60	-1290.63	136.30	20	-9.47	<.0001
type*temp	T	40	T	70	-822.89	136.30	20	-6.04	<.0001
type*temp	T	50	T	60	473.58	136.30	20	3.47	0.0024
type*temp	T	50	T	70	941.32	136.30	20	6.91	<.0001
type*temp	T	60	T	70	467.75	136.30	20	3.43	0.0026

Table 5.4: LS means for the “Duration of pectinase incubation” preliminary study

Least Squares Means							
Effect	type	duration	Estimate	Standard Error	DF	t Value	Pr > |t|
type*duration	C	1	2481.64	31.9480	12	77.68	<.0001
type*duration	C	2	2517.40	31.9480	12	78.80	<.0001
type*duration	C	3	2543.42	31.9480	12	79.61	<.0001
type*duration	T	1	3132.39	31.9480	12	98.05	<.0001

Least Squares Means							
Effect	type	duration	Estimate	Standard Error	DF	t Value	Pr > |t|
type*duration	T	2	3473.70	31.9480	12	108.73	<.0001
type*duration	T	3	3421.05	31.9480	12	107.08	<.0001

Differences of Least Squares Means									
Effect	type	duration	_type	_duration	Estimate	Standard Error	DF	t Value	Pr > |t|
type*duration	C	1	C	2	-35.7663	45.1813	12	-0.79	0.4440
type*duration	C	1	C	3	-61.7860	45.1813	12	-1.37	0.1965
type*duration	C	1	T	1	-650.76	45.1813	12	-14.40	<.0001
type*duration	C	1	T	2	-992.06	45.1813	12	-21.96	<.0001
type*duration	C	1	T	3	-939.41	45.1813	12	-20.79	<.0001
type*duration	C	2	C	3	-26.0197	45.1813	12	-0.58	0.5753
type*duration	C	2	T	1	-614.99	45.1813	12	-13.61	<.0001
type*duration	C	2	T	2	-956.29	45.1813	12	-21.17	<.0001
type*duration	C	2	T	3	-903.64	45.1813	12	-20.00	<.0001
type*duration	C	3	T	1	-588.97	45.1813	12	-13.04	<.0001
type*duration	C	3	T	2	-930.27	45.1813	12	-20.59	<.0001
type*duration	C	3	T	3	-877.62	45.1813	12	-19.42	<.0001
type*duration	T	1	T	2	-341.30	45.1813	12	-7.55	<.0001
type*duration	T	1	T	3	-288.65	45.1813	12	-6.39	<.0001
type*duration	T	2	T	3	52.6490	45.1813	12	1.17	0.2665

Appendix D. SAS output used for statistical analysis of response variables during PEF and pectinase treatments of grape pomace and peel

Table 5.5: LS means for the total polyphenols in the PEF extracts

Least Squares Means							
Effect	**variety**	**trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
variety*trt	flame	1	6544.52	11.0639	12	591.52	<.0001
variety*trt	flame	2	8102.52	11.0639	12	732.34	<.0001
variety*trt	frontena	1	2298.86	11.0639	12	207.78	<.0001
variety*trt	frontena	2	2865.79	11.0639	12	259.02	<.0001
variety*trt	stcroix	1	3640.26	11.0639	12	329.02	<.0001
variety*trt	stcroix	2	4662.49	11.0639	12	421.42	<.0001
variety*trt	stpepin	1	418.60	11.0639	12	37.83	<.0001
variety*trt	stpepin	2	467.06	11.0639	12	42.22	<.0001

Differences of Least Squares Means									
Effect	**variety**	**trt**	**_variety**	**_trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
variety*trt	flame	1	flame	2	-1558.00	15.6467	12	-99.57	<.0001
variety*trt	flame	1	frontena	1	4245.66	15.6467	12	271.35	<.0001
variety*trt	flame	1	frontena	2	3678.74	15.6467	12	235.11	<.0001
variety*trt	flame	1	stcroix	1	2904.27	15.6467	12	185.62	<.0001
variety*trt	flame	1	stcroix	2	1882.03	15.6467	12	120.28	<.0001
variety*trt	flame	1	stpepin	1	6125.93	15.6467	12	391.52	<.0001
variety*trt	flame	1	stpepin	2	6077.46	15.6467	12	388.42	<.0001
variety*trt	flame	2	frontena	1	5803.66	15.6467	12	370.92	<.0001
variety*trt	flame	2	frontena	2	5236.73	15.6467	12	334.69	<.0001
variety*trt	flame	2	stcroix	1	4462.26	15.6467	12	285.19	<.0001
variety*trt	flame	2	stcroix	2	3440.03	15.6467	12	219.86	<.0001
variety*trt	flame	2	stpepin	1	7683.92	15.6467	12	491.09	<.0001
variety*trt	flame	2	stpepin	2	7635.46	15.6467	12	487.99	<.0001
variety*trt	frontena	1	frontena	2	-566.92	15.6467	12	-36.23	<.0001
variety*trt	frontena	1	stcroix	1	-1341.40	15.6467	12	-85.73	<.0001
variety*trt	frontena	1	stcroix	2	-2363.63	15.6467	12	-151.06	<.0001
variety*trt	frontena	1	stpepin	1	1880.26	15.6467	12	120.17	<.0001
variety*trt	frontena	1	stpepin	2	1831.80	15.6467	12	117.07	<.0001
variety*trt	frontena	2	stcroix	1	-774.47	15.6467	12	-49.50	<.0001
variety*trt	frontena	2	stcroix	2	-1796.71	15.6467	12	-114.83	<.0001
variety*trt	frontena	2	stpepin	1	2447.19	15.6467	12	156.40	<.0001
variety*trt	frontena	2	stpepin	2	2398.72	15.6467	12	153.31	<.0001
variety*trt	stcroix	1	stcroix	2	-1022.24	15.6467	12	-65.33	<.0001
variety*trt	stcroix	1	stpepin	1	3221.66	15.6467	12	205.90	<.0001
variety*trt	stcroix	1	stpepin	2	3173.19	15.6467	12	202.80	<.0001

Differences of Least Squares Means									
Effect	**variety**	**trt**	**_variety**	**_trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
variety*trt	stcroix	2	stpepin	1	4243.90	15.6467	12	271.23	<.0001
variety*trt	stcroix	2	stpepin	2	4195.43	15.6467	12	268.14	<.0001
variety*trt	stpepin	1	stpepin	2	-48.4658	15.6467	12	-3.10	0.0092

Table 5.6: LS means for the total polyphenols in the pectinase extracts

Least Squares Means							
Effect	**variety**	**trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
variety*trt	flame	1	2898.51	8.7730	12	330.39	<.0001
variety*trt	flame	2	3378.03	8.7730	12	385.05	<.0001
variety*trt	frontena	1	1212.88	8.7730	12	138.25	<.0001
variety*trt	frontena	2	1814.52	8.7730	12	206.83	<.0001
variety*trt	stcroix	1	2884.41	8.7730	12	328.78	<.0001
variety*trt	stcroix	2	3199.00	8.7730	12	364.64	<.0001
variety*trt	stpepin	1	151.21	8.7730	12	17.24	<.0001
variety*trt	stpepin	2	203.12	8.7730	12	23.15	<.0001

Differences of Least Squares Means									
Effect	**variety**	**trt**	**_variety**	**_trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
variety*trt	flame	1	flame	2	-479.52	12.4069	12	-38.65	<.0001
variety*trt	flame	1	frontena	1	1685.63	12.4069	12	135.86	<.0001
variety*trt	flame	1	frontena	2	1083.98	12.4069	12	87.37	<.0001
variety*trt	flame	1	stcroix	1	14.0977	12.4069	12	1.14	0.2780
variety*trt	flame	1	stcroix	2	-300.49	12.4069	12	-24.22	<.0001
variety*trt	flame	1	stpepin	1	2747.30	12.4069	12	221.43	<.0001
variety*trt	flame	1	stpepin	2	2695.38	12.4069	12	217.25	<.0001
variety*trt	flame	2	frontena	1	2165.15	12.4069	12	174.51	<.0001
variety*trt	flame	2	frontena	2	1563.51	12.4069	12	126.02	<.0001
variety*trt	flame	2	stcroix	1	493.62	12.4069	12	39.79	<.0001
variety*trt	flame	2	stcroix	2	179.03	12.4069	12	14.43	<.0001
variety*trt	flame	2	stpepin	1	3226.82	12.4069	12	260.08	<.0001
variety*trt	flame	2	stpepin	2	3174.91	12.4069	12	255.90	<.0001
variety*trt	frontena	1	frontena	2	-601.65	12.4069	12	-48.49	<.0001
variety*trt	frontena	1	stcroix	1	-1671.53	12.4069	12	-134.73	<.0001
variety*trt	frontena	1	stcroix	2	-1986.12	12.4069	12	-160.08	<.0001
variety*trt	frontena	1	stpepin	1	1061.67	12.4069	12	85.57	<.0001
variety*trt	frontena	1	stpepin	2	1009.76	12.4069	12	81.39	<.0001
variety*trt	frontena	2	stcroix	1	-1069.89	12.4069	12	-86.23	<.0001
variety*trt	frontena	2	stcroix	2	-1384.47	12.4069	12	-111.59	<.0001
variety*trt	frontena	2	stpepin	1	1663.31	12.4069	12	134.06	<.0001
variety*trt	frontena	2	stpepin	2	1611.40	12.4069	12	129.88	<.0001
variety*trt	stcroix	1	stcroix	2	-314.59	12.4069	12	-25.36	<.0001
variety*trt	stcroix	1	stpepin	1	2733.20	12.4069	12	220.30	<.0001

Differences of Least Squares Means									
Effect	**variety**	**trt**	**_variety**	**_trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
variety*trt	stcroix	1	stpepin	2	2681.29	12.4069	12	216.11	<.0001
variety*trt	stcroix	2	stpepin	1	3047.79	12.4069	12	245.65	<.0001
variety*trt	stcroix	2	stpepin	2	2995.87	12.4069	12	241.47	<.0001
variety*trt	stpepin	1	stpepin	2	-51.9119	12.4069	12	-4.18	0.0013

Table 5.7: Estimates and LS means for the HPLC analysis of the Frontenac PEF extract

Estimates					
Label	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
GAc vs GAp	-1.2559	2.2790	16	-0.55	0.5892
Catc vs Catp	-13.8336	2.2790	16	-6.07	<.0001
Rutc vs Rutp	-2.0978	2.2790	16	-0.92	0.3710
Myrc vs Myrp	-25.8894	2.2790	16	-11.36	<.0001

Least Squares Means						
Effect	**phenoltrt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
phenoltrt	Catc	29.2146	1.6115	16	18.13	<.0001
phenoltrt	Catp	43.0482	1.6115	16	26.71	<.0001
phenoltrt	GAc	0.7520	1.6115	16	0.47	0.6470
phenoltrt	GAp	2.0079	1.6115	16	1.25	0.2307
phenoltrt	Myrc	0	1.6115	16	0.00	1.0000
phenoltrt	Myrp	25.8894	1.6115	16	16.07	<.0001
phenoltrt	Rutc	7.6281	1.6115	16	4.73	0.0002
phenoltrt	Rutp	9.7260	1.6115	16	6.04	<.0001

Table 5.8: Estimates and LS means for the HPLC analysis of the Frontenac pectinase extract

Estimates					
Label	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
GAc vs GApec	-0.05770	1.8129	16	-0.03	0.9750
Catc vs Catpec	-5.3196	1.8129	16	-2.93	0.0097
Myrc vs Myrpec	-0.04330	1.8129	16	-0.02	0.9812
Epicatc vs Epicatpec	-9.8139	1.8129	16	-5.41	<.0001

Least Squares Means						
Effect	**phenoltrt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
phenoltrt	Catc	15.3935	1.2819	16	12.01	<.0001
phenoltrt	Catpec	20.7131	1.2819	16	16.16	<.0001
phenoltrt	Epicatc	25.6926	1.2819	16	20.04	<.0001
phenoltrt	Epicatpe	35.5065	1.2819	16	27.70	<.0001
phenoltrt	GAc	0.06337	1.2819	16	0.05	0.9612
phenoltrt	GApec	0.1211	1.2819	16	0.09	0.9259
phenoltrt	Myrc	24.9956	1.2819	16	19.50	<.0001

Least Squares Means						
Effect	**phenoltrt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
phenoltrt	Myrpec	25.0389	1.2819	16	19.53	<.0001

Table 5.8: Estimates and LS means for the HPLC analysis of the St.Croix PEF extract

Estimates					
Label	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
GAc vs GAp	-0.6407	5.5833	20	-0.11	0.9098
Catc vs Catp	-23.7272	5.5833	20	-4.25	0.0004
Rutc vs Rutp	2.5852	5.5833	20	0.46	0.6483
Epicatc vs Epicatp	-14.0978	5.5833	20	-2.52	0.0201
Querc vs Querp	-22.2250	5.5833	20	-3.98	0.0007

Least Squares Means						
Effect	**phenoltrt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
phenoltrt	Catc	37.7193	3.9480	20	9.55	<.0001
phenoltrt	Catp	61.4464	3.9480	20	15.56	<.0001
phenoltrt	Epicatc	38.7261	3.9480	20	9.81	<.0001
phenoltrt	Epicatp	52.8238	3.9480	20	13.38	<.0001
phenoltrt	GAc	0.07723	3.9480	20	0.02	0.9846
phenoltrt	GAp	0.7179	3.9480	20	0.18	0.8575
phenoltrt	Querc	3.55E-15	3.9480	20	0.00	1.0000
phenoltrt	Querp	22.2250	3.9480	20	5.63	<.0001
phenoltrt	Rutc	22.9124	3.9480	20	5.80	<.0001
phenoltrt	Rutp	20.3272	3.9480	20	5.15	<.0001

Table 5.9: Estimates and LS means for the HPLC analysis of the St.Croix pectinase extract

Estimates					
Label	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
GAc vs GApec	-2.8749	3.9555	15	-0.73	0.4785
Catc vs Catpec	-25.9675	3.9555	15	-6.56	<.0001
Querc vs Quercpec	-22.2925	4.4224	15	-5.04	0.0001
Epicatc vs Epicatpec	-20.8650	3.9555	15	-5.27	<.0001

Least Squares Means						
Effect	**phenoltrt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
phenoltrt	Catc	3.55E-15	2.7969	15	0.00	1.0000
phenoltrt	Catpec	25.9675	2.7969	15	9.28	<.0001
phenoltrt	Epicatc	27.1063	2.7969	15	9.69	<.0001
phenoltrt	Epicatpe	47.9713	2.7969	15	17.15	<.0001
phenoltrt	GAc	0.05327	2.7969	15	0.02	0.9851
phenoltrt	GApec	2.9282	2.7969	15	1.05	0.3117
phenoltrt	Querc	0	2.7969	15	0.00	1.0000

Least Squares Means						
Effect	**phenoltrt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
phenoltrt	croix	22.2925	3.4255	15	6.51	<.0001

Table 5.10: Estimates and LS means for the HPLC analysis of the St.Pepin PEF extract

Estimates					
Label	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
GAc vs GAp	-0.4063	1.0471	8	-0.39	0.7081
Catc vs Catp	-5.8215	1.0471	8	-5.56	0.0005

Least Squares Means						
Effect	**phenoltrt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
phenoltrt	Catc	28.4816	0.7404	8	38.47	<.0001
phenoltrt	Catp	34.3031	0.7404	8	46.33	<.0001
phenoltrt	GAc	0.4157	0.7404	8	0.56	0.5898
phenoltrt	GAp	0.8220	0.7404	8	1.11	0.2992

Table 5.11: Estimates and LS means for the HPLC analysis of the St.Pepin pectinase extract

Estimates					
Label	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
GAc vs GApec	-1.0807	4.6871	8	-0.23	0.8234
Catc vs Catpec	-4.9545	4.6871	8	-1.06	0.3214

Least Squares Means						
Effect	**phenoltrt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
phenoltrt	Catc	11.1648	3.3142	8	3.37	0.0098
phenoltrt	Catpec	16.1193	3.3142	8	4.86	0.0013
phenoltrt	GAc	2.22E-16	3.3142	8	0.00	1.0000
phenoltrt	GApec	1.0807	3.3142	8	0.33	0.7527

Table 5.12: Estimates and LS means for the HPLC analysis of the Flame peel PEF extract

Estimates					
Label	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
GAc vs GAp	-2.7145	4.5417	16	-0.60	0.5584
Catc vs Catp	-13.1795	4.5417	16	-2.90	0.0104
Rutc vs Rutp	-16.9994	4.5417	16	-3.74	0.0018
Myrc vs Myrp	-26.1965	4.5417	16	-5.77	<.0001

Least Squares Means

Effect	phenoltrt	Estimate	Standard Error	DF	t Value	Pr > |t|
phenoltrt	Catc	37.1833	3.2115	16	11.58	<.0001
phenoltrt	Catp	50.3628	3.2115	16	15.68	<.0001
phenoltrt	GAc	0.7037	3.2115	16	0.22	0.8293
phenoltrt	GAp	3.4182	3.2115	16	1.06	0.3030
phenoltrt	Myrc	0	3.2115	16	0.00	1.0000
phenoltrt	Myrp	26.1965	3.2115	16	8.16	<.0001
phenoltrt	Rutc	22.5654	3.2115	16	7.03	<.0001
phenoltrt	Rutp	39.5648	3.2115	16	12.32	<.0001

Table 5.13: Estimates and LS means for the HPLC analysis of the Flame peel pectinase extract

Estimates					
Label	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
GAc vs GApec	-2.0934	0.2625	24	-7.97	<.0001
Catc vs Catpec	-3.1275	0.2625	24	-11.91	<.0001
Rutc vs Rutpec	-0.6033	0.2625	24	-2.30	0.0306
Myrc vs Myrpec	-0.4616	0.2625	24	-1.76	0.0914
Resc vs Respec	-0.00360	0.2625	24	-0.01	0.9892
Epicatc vs Epicatpec	-5.5496	0.2625	24	-21.14	<.0001

Least Squares Means						
Effect	**phenoltrt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
phenoltrt	Catc	29.9899	0.1856	24	161.56	<.0001
phenoltrt	Catpec	33.1174	0.1856	24	178.40	<.0001
phenoltrt	Epicatc	26.8264	0.1856	24	144.51	<.0001
phenoltrt	Epicatpe	32.3760	0.1856	24	174.41	<.0001
phenoltrt	GAc	0.1758	0.1856	24	0.95	0.3531
phenoltrt	GApec	2.2692	0.1856	24	12.22	<.0001
phenoltrt	Myrc	25.4609	0.1856	24	137.16	<.0001
phenoltrt	Myrpec	25.9225	0.1856	24	139.64	<.0001
phenoltrt	Resc	15.8128	0.1856	24	85.18	<.0001
phenoltrt	Respec	15.8164	0.1856	24	85.20	<.0001
phenoltrt	Rutc	8.0958	0.1856	24	43.61	<.0001
phenoltrt	Rutpec	8.6991	0.1856	24	46.86	<.0001

Table 5.14: LS means for the FRAP analysis of the PEF extracts

Least Squares Means							
Effect	**variety**	**trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
variety*trt	flame	1	779.81	6.9740	12	111.82	<.0001
variety*trt	flame	2	1064.78	6.9740	12	152.68	<.0001
variety*trt	frontena	1	1093.08	6.9740	12	156.74	<.0001
variety*trt	frontena	2	1808.63	6.9740	12	259.34	<.0001
variety*trt	stcroix	1	1213.39	6.9740	12	173.99	<.0001
variety*trt	stcroix	2	1576.90	6.9740	12	226.11	<.0001
variety*trt	stpepin	1	35.0181	6.9740	12	5.02	0.0003
variety*trt	stpepin	2	39.9222	6.9740	12	5.72	<.0001

Differences of Least Squares Means									
Effect	**variety**	**trt**	**_variety**	**_trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
variety*trt	flame	1	flame	2	-284.97	9.8628	12	-28.89	<.0001
variety*trt	flame	1	frontena	1	-313.28	9.8628	12	-31.76	<.0001
variety*trt	flame	1	frontena	2	-1028.82	9.8628	12	-104.31	<.0001
variety*trt	flame	1	stcroix	1	-433.58	9.8628	12	-43.96	<.0001
variety*trt	flame	1	stcroix	2	-797.10	9.8628	12	-80.82	<.0001
variety*trt	flame	1	stpepin	1	744.79	9.8628	12	75.51	<.0001
variety*trt	flame	1	stpepin	2	739.88	9.8628	12	75.02	<.0001
variety*trt	flame	2	frontena	1	-28.3056	9.8628	12	-2.87	0.0141
variety*trt	flame	2	frontena	2	-743.85	9.8628	12	-75.42	<.0001
variety*trt	flame	2	stcroix	1	-148.61	9.8628	12	-15.07	<.0001
variety*trt	flame	2	stcroix	2	-512.13	9.8628	12	-51.93	<.0001
variety*trt	flame	2	stpepin	1	1029.76	9.8628	12	104.41	<.0001
variety*trt	flame	2	stpepin	2	1024.86	9.8628	12	103.91	<.0001
variety*trt	frontena	1	frontena	2	-715.54	9.8628	12	-72.55	<.0001
variety*trt	frontena	1	stcroix	1	-120.31	9.8628	12	-12.20	<.0001
variety*trt	frontena	1	stcroix	2	-483.82	9.8628	12	-49.06	<.0001
variety*trt	frontena	1	stpepin	1	1058.07	9.8628	12	107.28	<.0001
variety*trt	frontena	1	stpepin	2	1053.16	9.8628	12	106.78	<.0001
variety*trt	frontena	2	stcroix	1	595.24	9.8628	12	60.35	<.0001
variety*trt	frontena	2	stcroix	2	231.72	9.8628	12	23.49	<.0001
variety*trt	frontena	2	stpepin	1	1773.61	9.8628	12	179.83	<.0001
variety*trt	frontena	2	stpepin	2	1768.70	9.8628	12	179.33	<.0001
variety*trt	stcroix	1	stcroix	2	-363.51	9.8628	12	-36.86	<.0001
variety*trt	stcroix	1	stpepin	1	1178.37	9.8628	12	119.48	<.0001
variety*trt	stcroix	1	stpepin	2	1173.47	9.8628	12	118.98	<.0001
variety*trt	stcroix	2	stpepin	1	1541.88	9.8628	12	156.33	<.0001
variety*trt	stcroix	2	stpepin	2	1536.98	9.8628	12	155.84	<.0001
variety*trt	stpepin	1	stpepin	2	-4.9042	9.8628	12	-0.50	0.6280

Table 5.15: LS means for the FRAP analysis of the Pectinase extracts

Least Squares Means							
Effect	**variety**	**trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
variety*trt	flame	1	228.29	8.7742	12	26.02	<.0001
variety*trt	flame	2	554.31	8.7742	12	63.17	<.0001
variety*trt	frontena	1	349.43	8.7742	12	39.82	<.0001
variety*trt	frontena	2	595.29	8.7742	12	67.85	<.0001
variety*trt	stcroix	1	848.47	8.7742	12	96.70	<.0001
variety*trt	stcroix	2	1345.17	8.7742	12	153.31	<.0001
variety*trt	stpepin	1	1.8042	8.7742	12	0.21	0.8405
variety*trt	stpepin	2	2.6750	8.7742	12	0.30	0.7657

Differences of Least Squares Means									
Effect	**variety**	**trt**	**_variety**	**_trt**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
variety*trt	flame	1	flame	2	-326.01	12.4086	12	-26.27	<.0001
variety*trt	flame	1	frontena	1	-121.14	12.4086	12	-9.76	<.0001
variety*trt	flame	1	frontena	2	-367.00	12.4086	12	-29.58	<.0001
variety*trt	flame	1	stcroix	1	-620.18	12.4086	12	-49.98	<.0001
variety*trt	flame	1	stcroix	2	-1116.87	12.4086	12	-90.01	<.0001
variety*trt	flame	1	stpepin	1	226.49	12.4086	12	18.25	<.0001
variety*trt	flame	1	stpepin	2	225.62	12.4086	12	18.18	<.0001
variety*trt	flame	2	frontena	1	204.88	12.4086	12	16.51	<.0001
variety*trt	flame	2	frontena	2	-40.9861	12.4086	12	-3.30	0.0063
variety*trt	flame	2	stcroix	1	-294.17	12.4086	12	-23.71	<.0001
variety*trt	flame	2	stcroix	2	-790.86	12.4086	12	-63.74	<.0001
variety*trt	flame	2	stpepin	1	552.50	12.4086	12	44.53	<.0001
variety*trt	flame	2	stpepin	2	551.63	12.4086	12	44.46	<.0001
variety*trt	frontena	1	frontena	2	-245.86	12.4086	12	-19.81	<.0001
variety*trt	frontena	1	stcroix	1	-499.04	12.4086	12	-40.22	<.0001
variety*trt	frontena	1	stcroix	2	-995.74	12.4086	12	-80.25	<.0001
variety*trt	frontena	1	stpepin	1	347.63	12.4086	12	28.02	<.0001
variety*trt	frontena	1	stpepin	2	346.76	12.4086	12	27.94	<.0001
variety*trt	frontena	2	stcroix	1	-253.18	12.4086	12	-20.40	<.0001
variety*trt	frontena	2	stcroix	2	-749.87	12.4086	12	-60.43	<.0001
variety*trt	frontena	2	stpepin	1	593.49	12.4086	12	47.83	<.0001
variety*trt	frontena	2	stpepin	2	592.62	12.4086	12	47.76	<.0001
variety*trt	stcroix	1	stcroix	2	-496.69	12.4086	12	-40.03	<.0001
variety*trt	stcroix	1	stpepin	1	846.67	12.4086	12	68.23	<.0001
variety*trt	stcroix	1	stpepin	2	845.80	12.4086	12	68.16	<.0001
variety*trt	stcroix	2	stpepin	1	1343.36	12.4086	12	108.26	<.0001
variety*trt	stcroix	2	stpepin	2	1342.49	12.4086	12	108.19	<.0001
variety*trt	stpepin	1	stpepin	2	-0.8708	12.4086	12	-0.07	0.9452

Appendix E. HPLC chromatograms

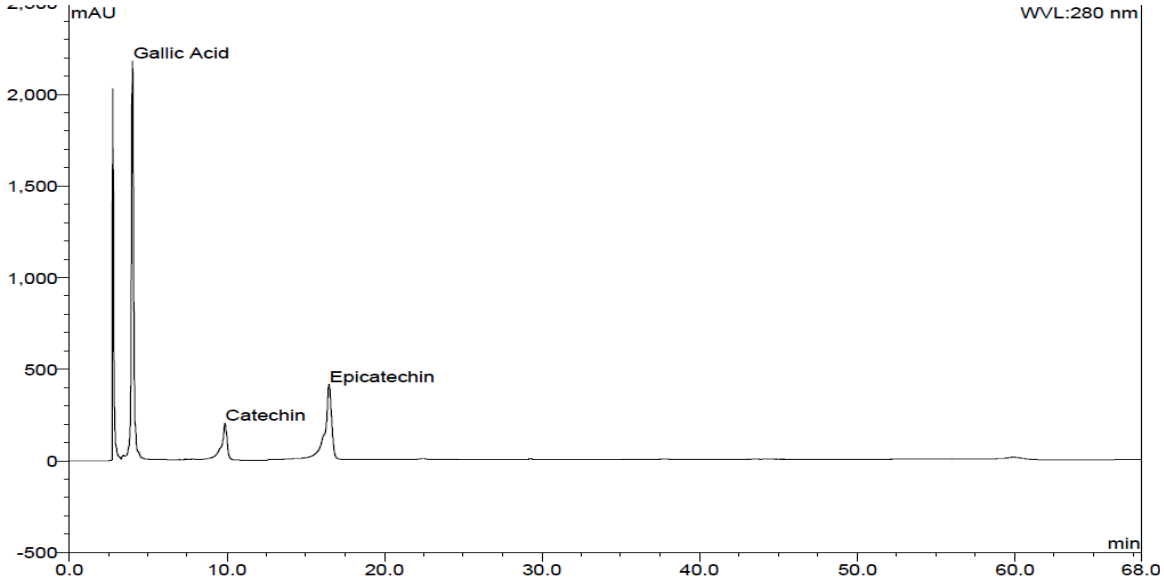


Fig 5.1: HPLC chromatogram for the standards at λ = 280 nm

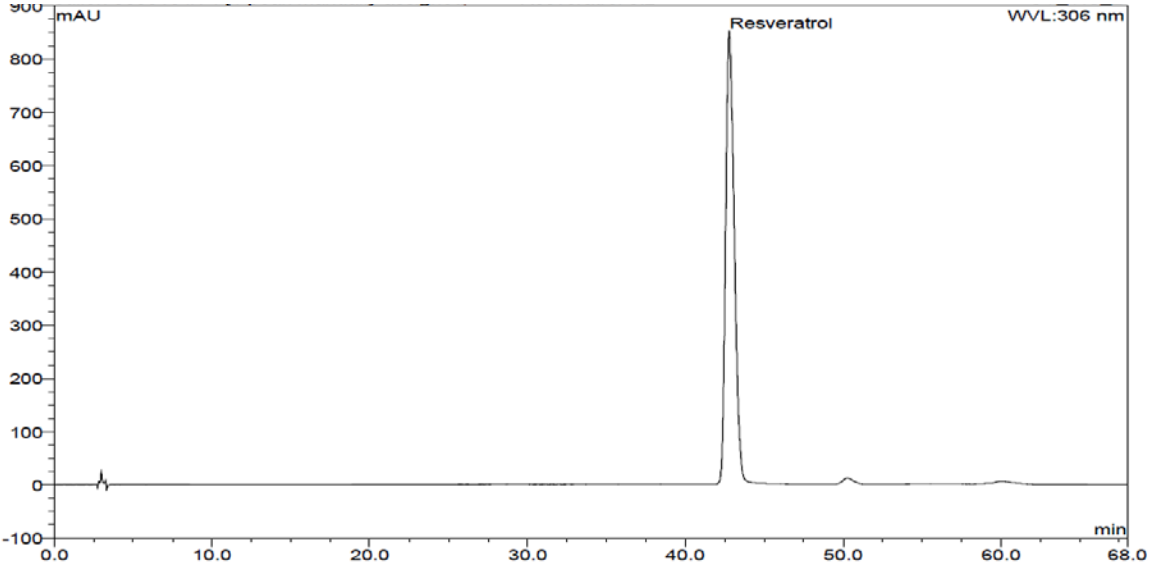


Fig 5.2: HPLC chromatogram for the standards at λ = 306 nm

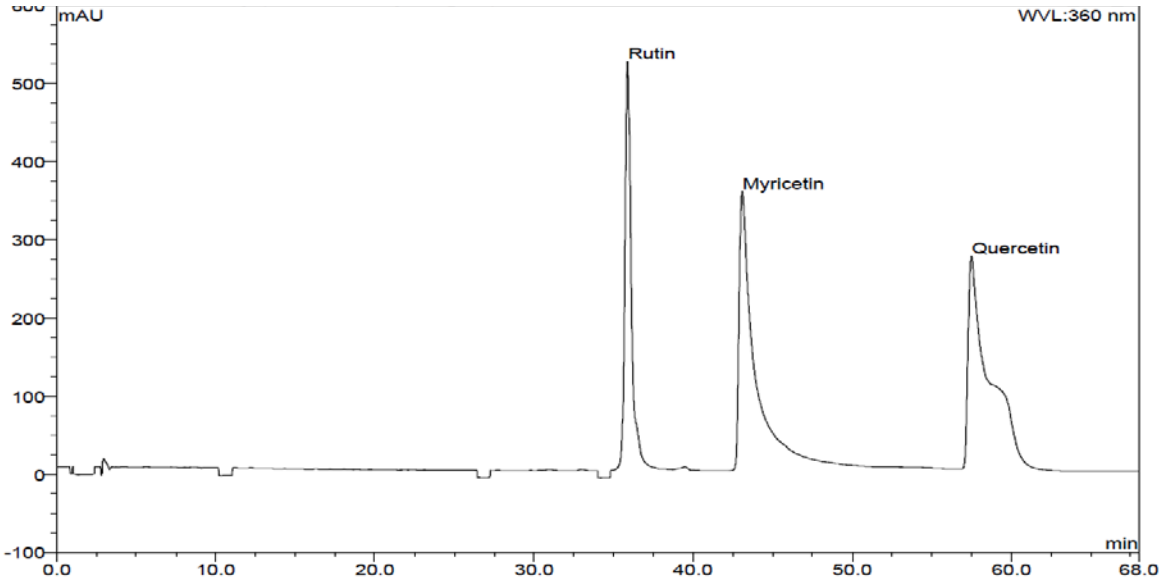
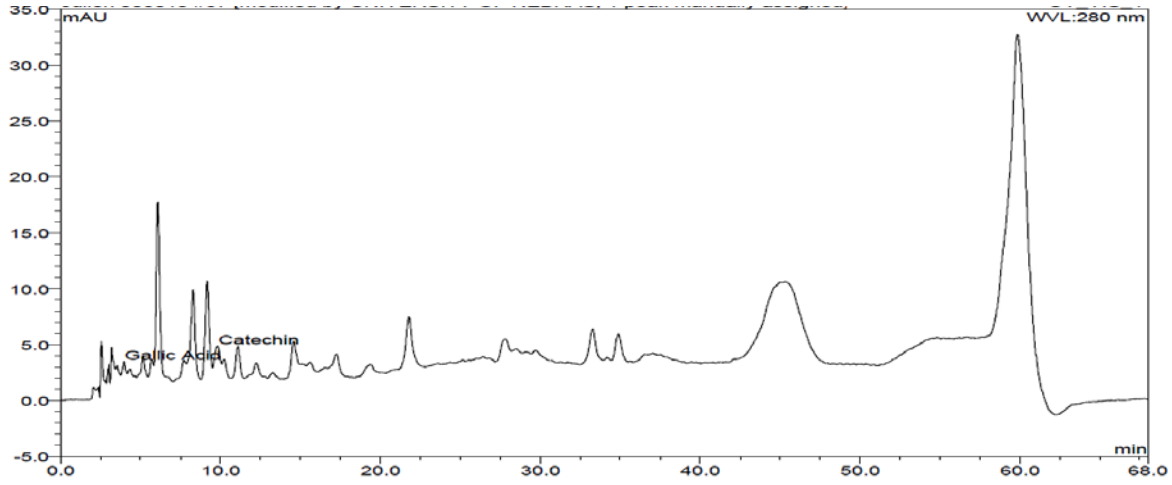
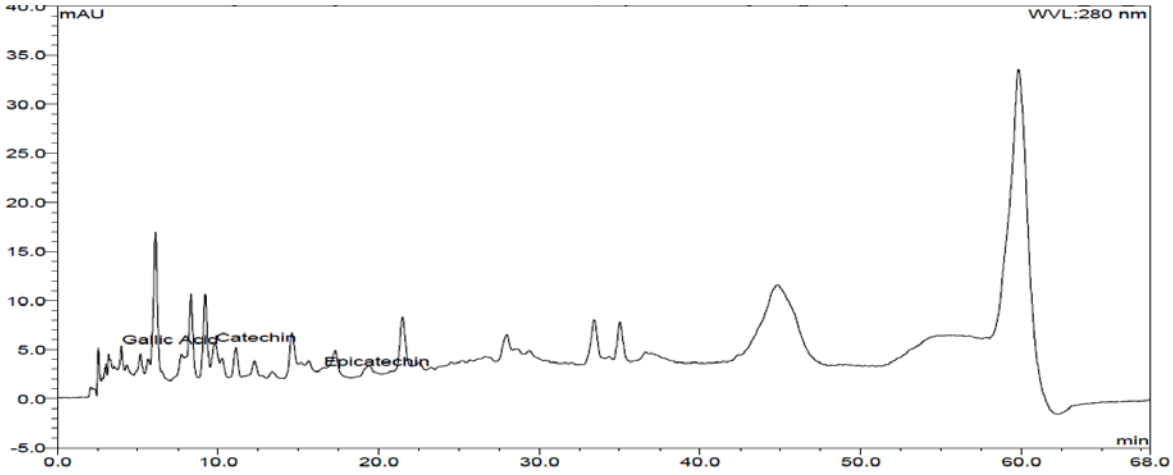


Fig 5.3: HPLC chromatogram for the standards at λ = 360 nm

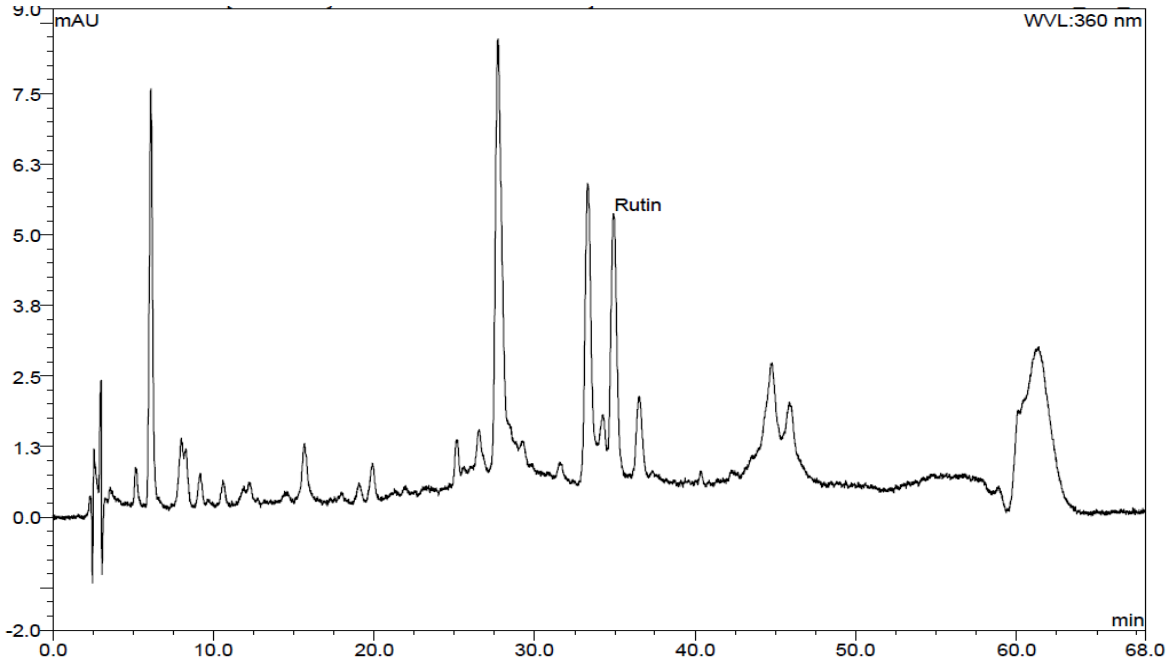


a. Control (No PEF)

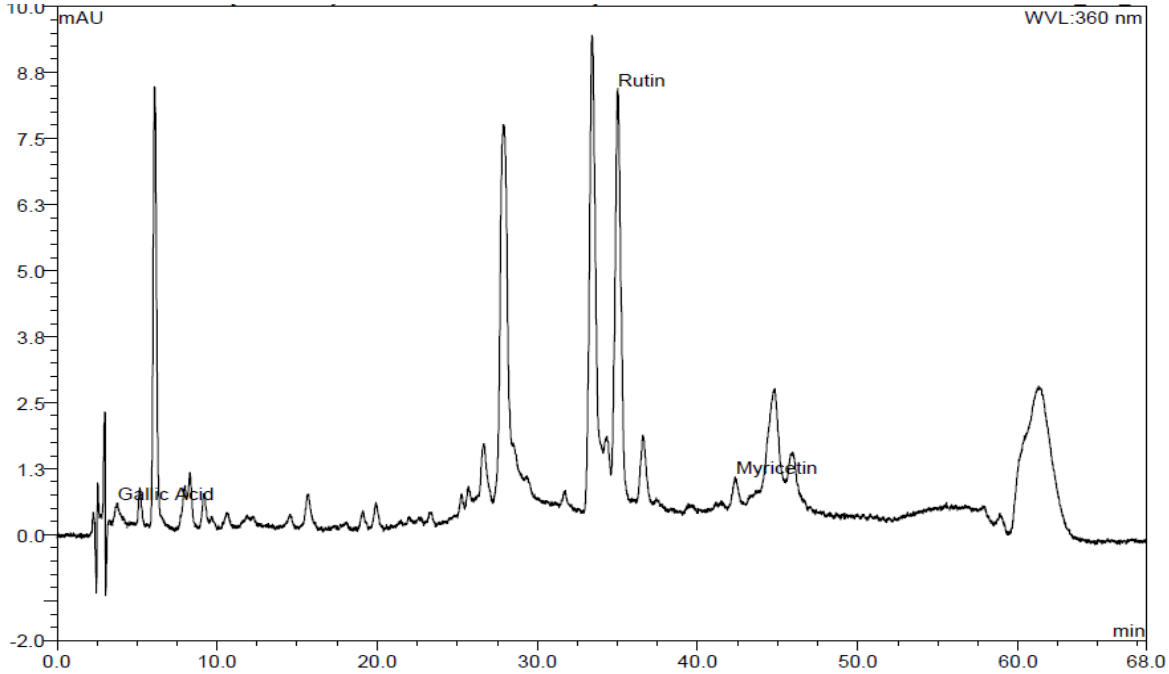


b. PEF treatment

Fig 5.4: Chilean Flame peel HPLC chromatograms for the detection of Gallic acid, catechin and Epicatechin at λ=280 nm for the PEF treatment

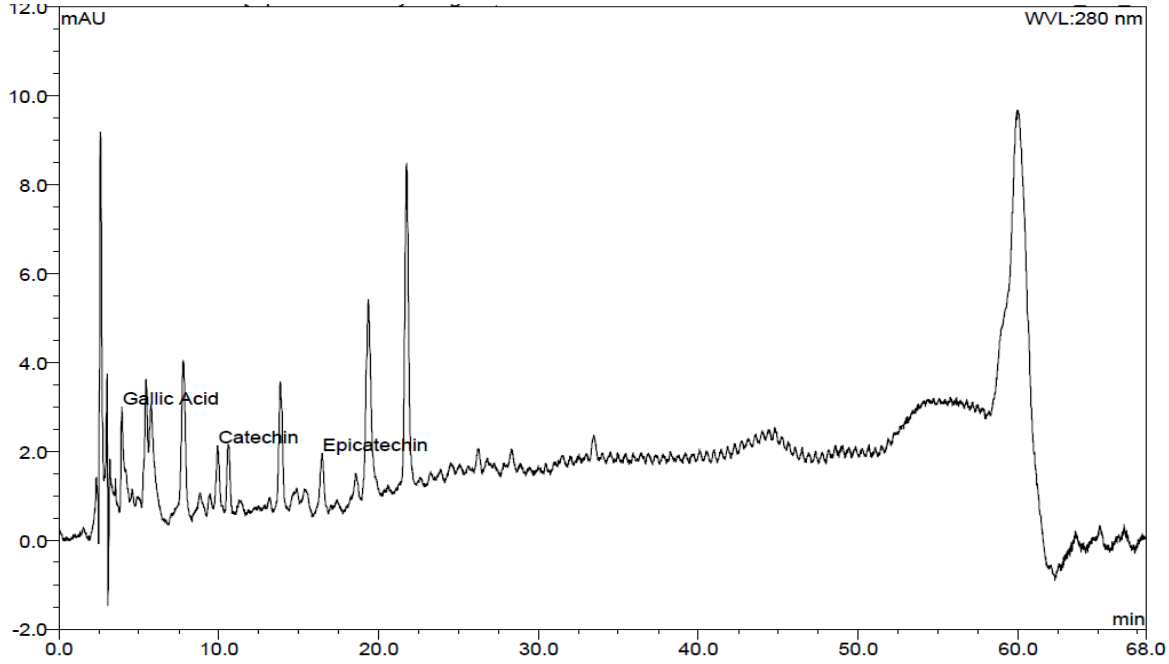


a. Control (No PEF)

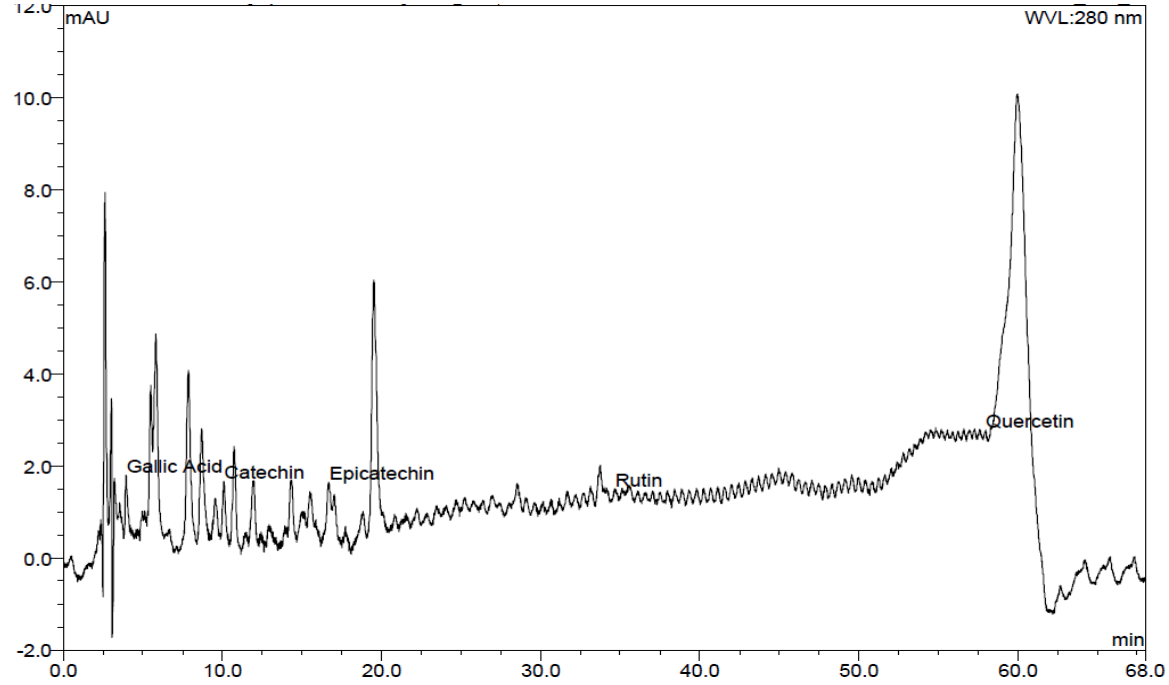


b. PEF treatment

Fig 5.5: Chilean Flame peel HPLC chromatograms for the detection of Rutin, Quercetin and Myricetin at λ=360 nm for the PEF treatment

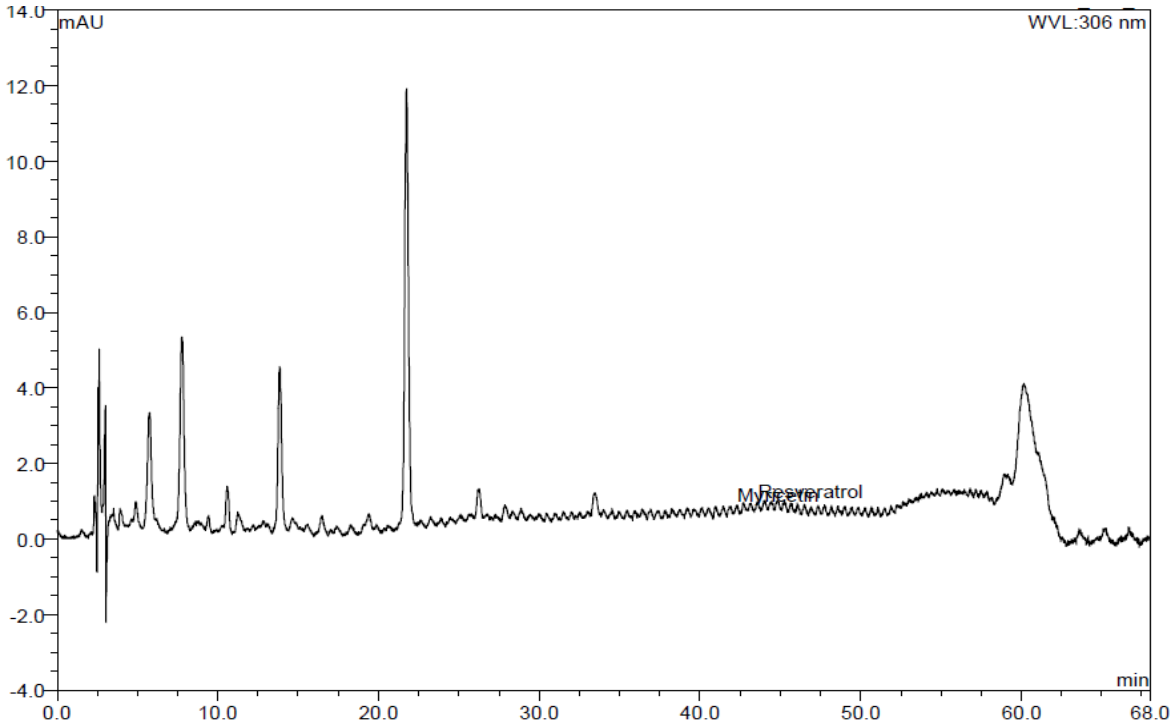


a. Control (No pectinase)

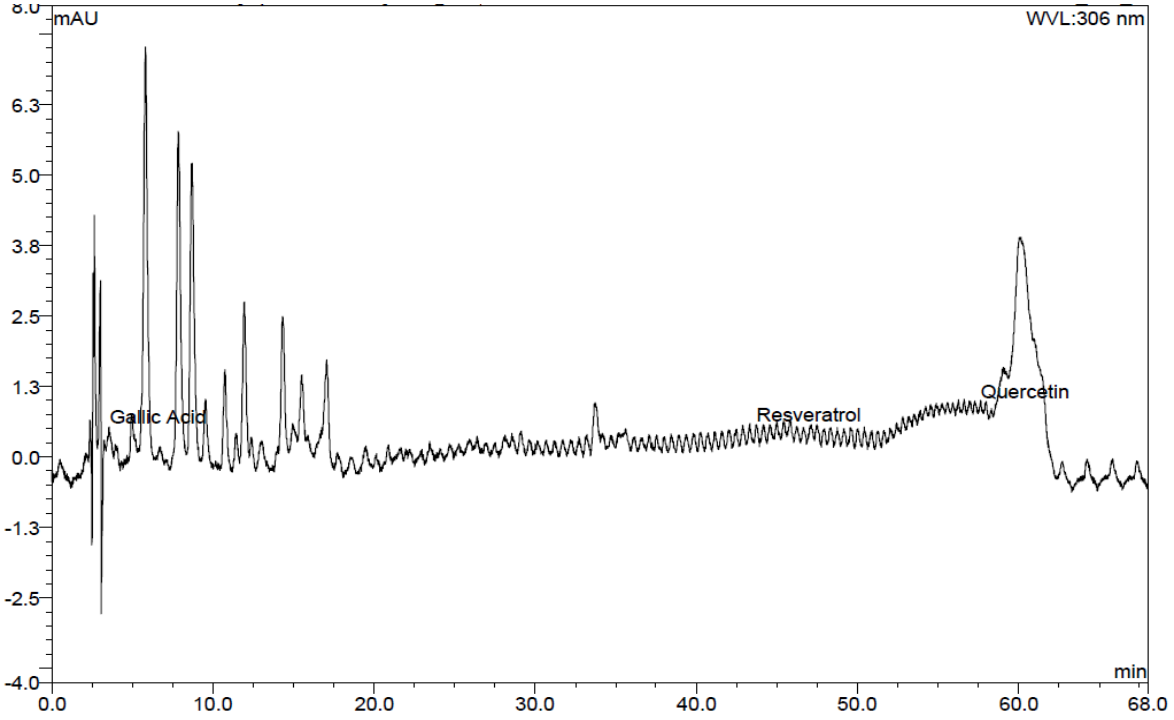


b. Pectinase treatment

Fig 5.6: Chilean Flame peel HPLC chromatograms for the detection of Gallic acid, Catechin and Epicatechin at λ=280 nm for the pectinase treatment

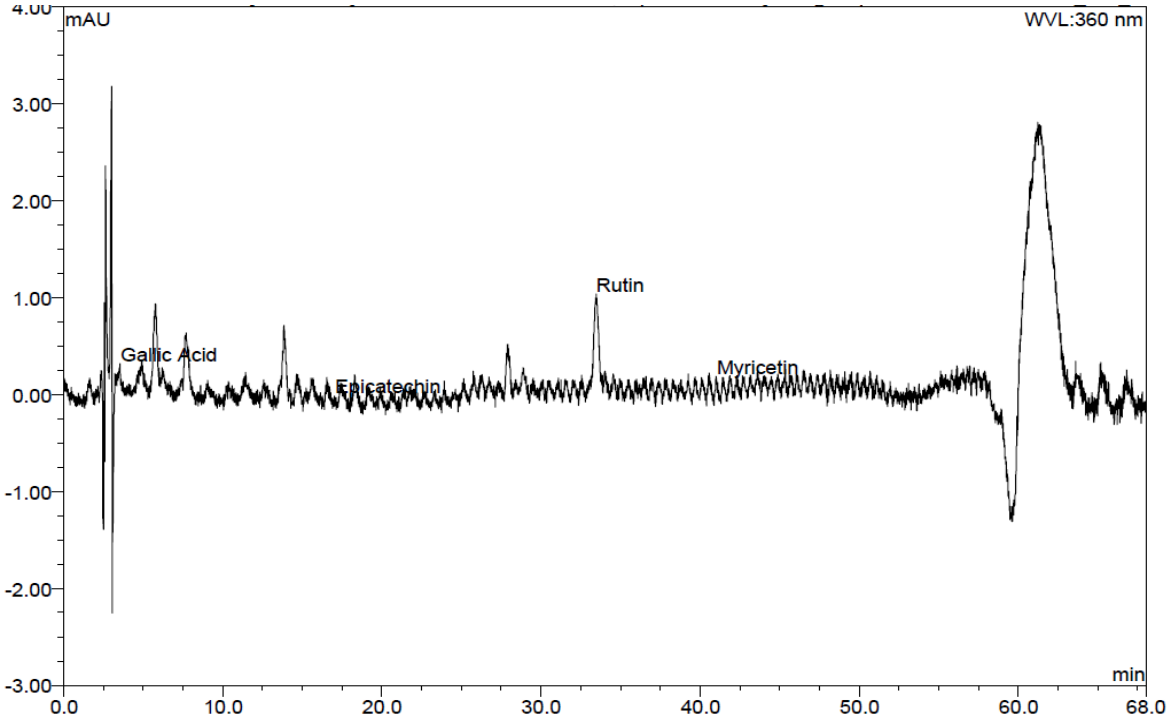


a. Control (No Pectinase)

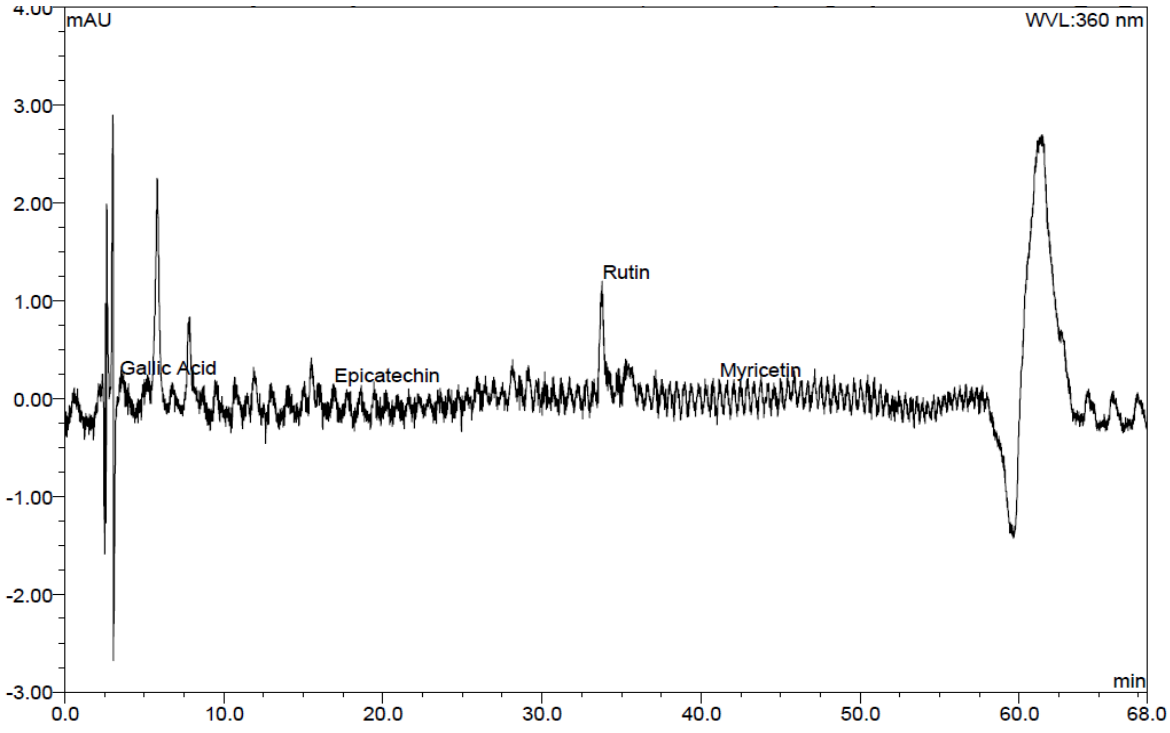


b. Pectinase treatment

Fig 5.7: Chilean Flame peel HPLC chromatograms for the detection of Resveratrol at λ=280 nm for the pectinase treatment

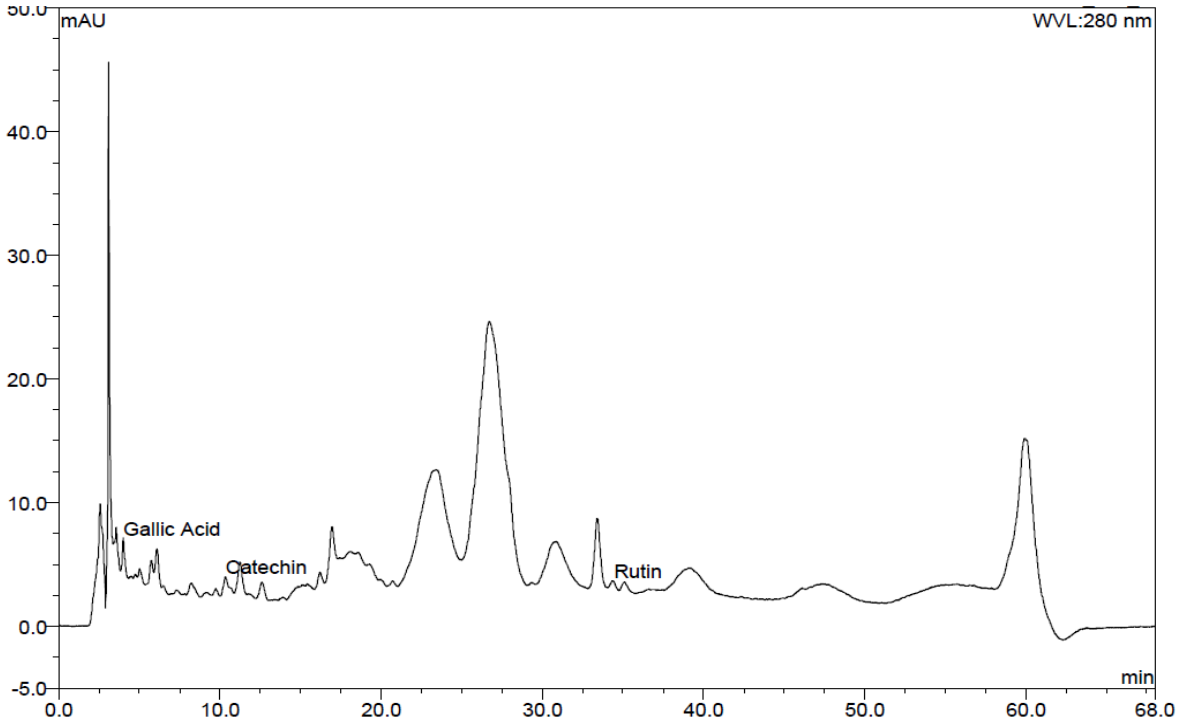


a. Control (No pectinase)

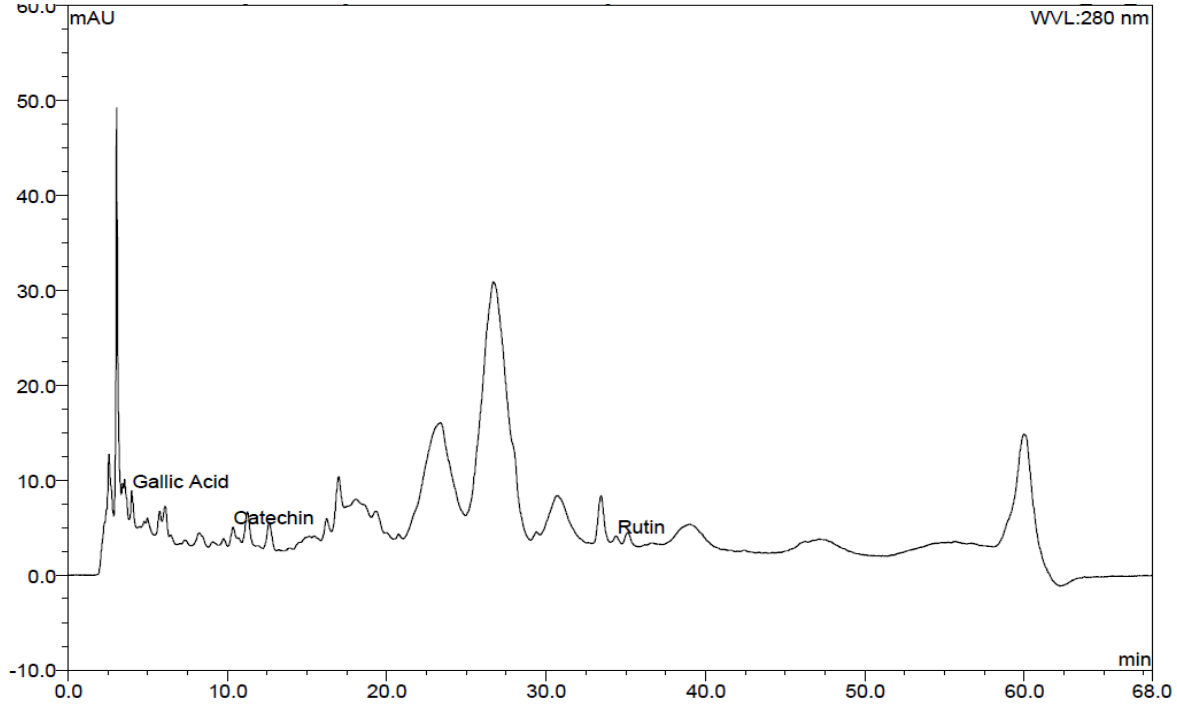


b. Pectinase treatment

Fig 5.7: Chilean Flame peel HPLC chromatograms for the detection of Rutin, Quercetin and Myricetin at λ=280 nm for the pectinase treatment

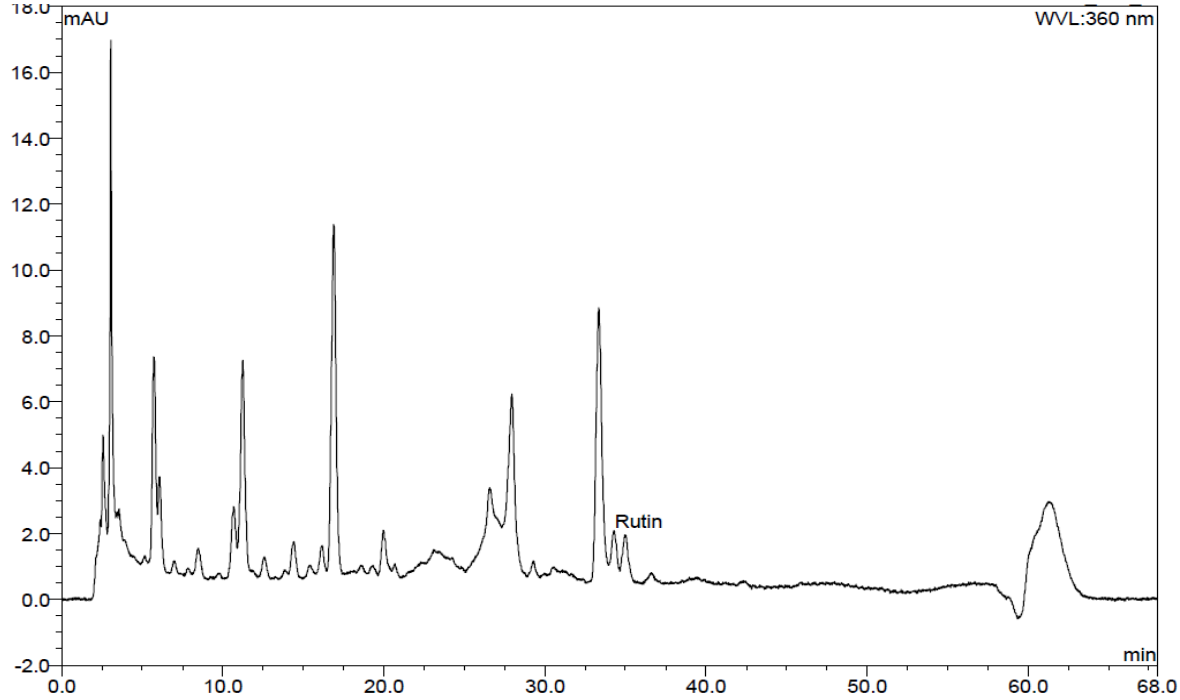


a. Control (No PEF)

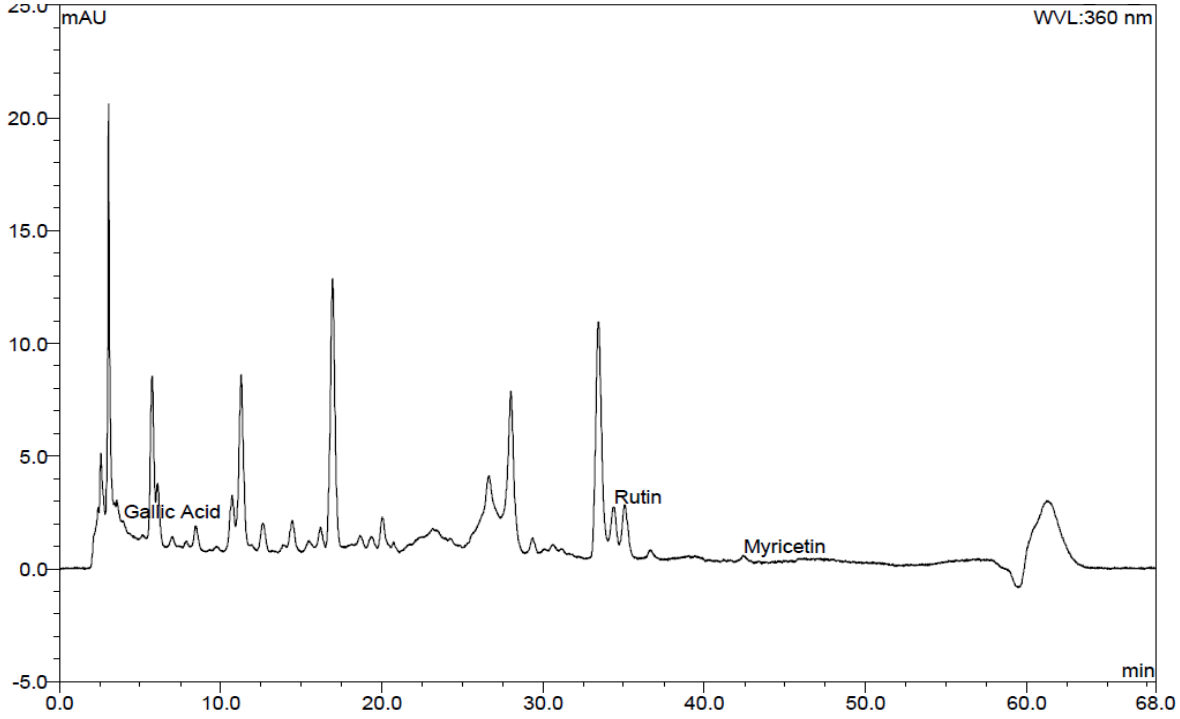


b. PEF treatment

Fig 5.8: Frontenac pomace HPLC chromatograms for the detection of Gallic acid, Catechin and Epicatechin at λ=280 nm for the PEF treatment

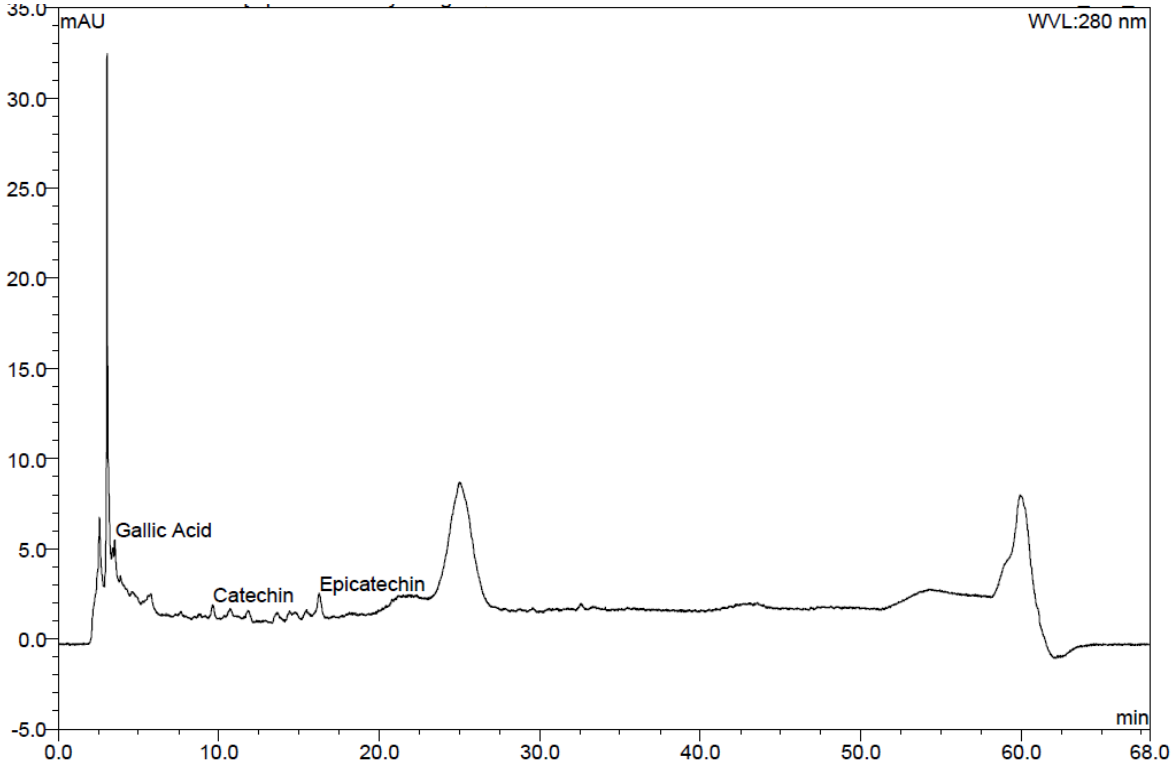


a. Control (No PEF)

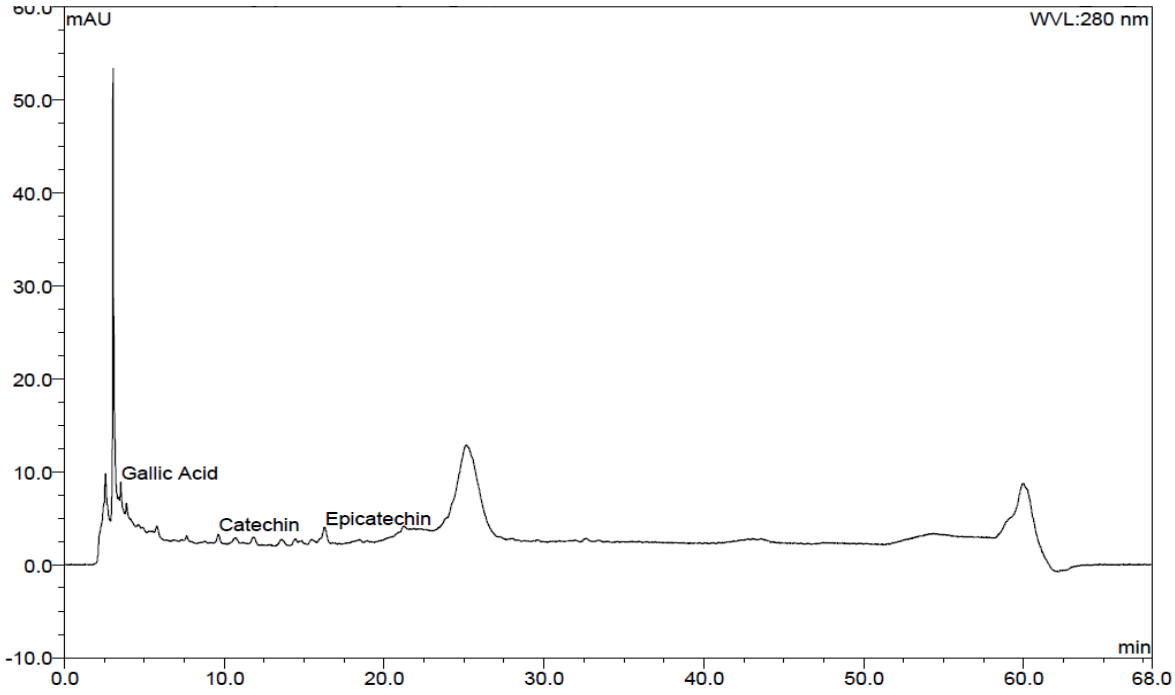


b. PEF treatment

Fig 5.9: Frontenac pomace HPLC chromatograms for the detection of Rutin, Quercetin and Myricetin at λ=360 nm for the PEF treatment

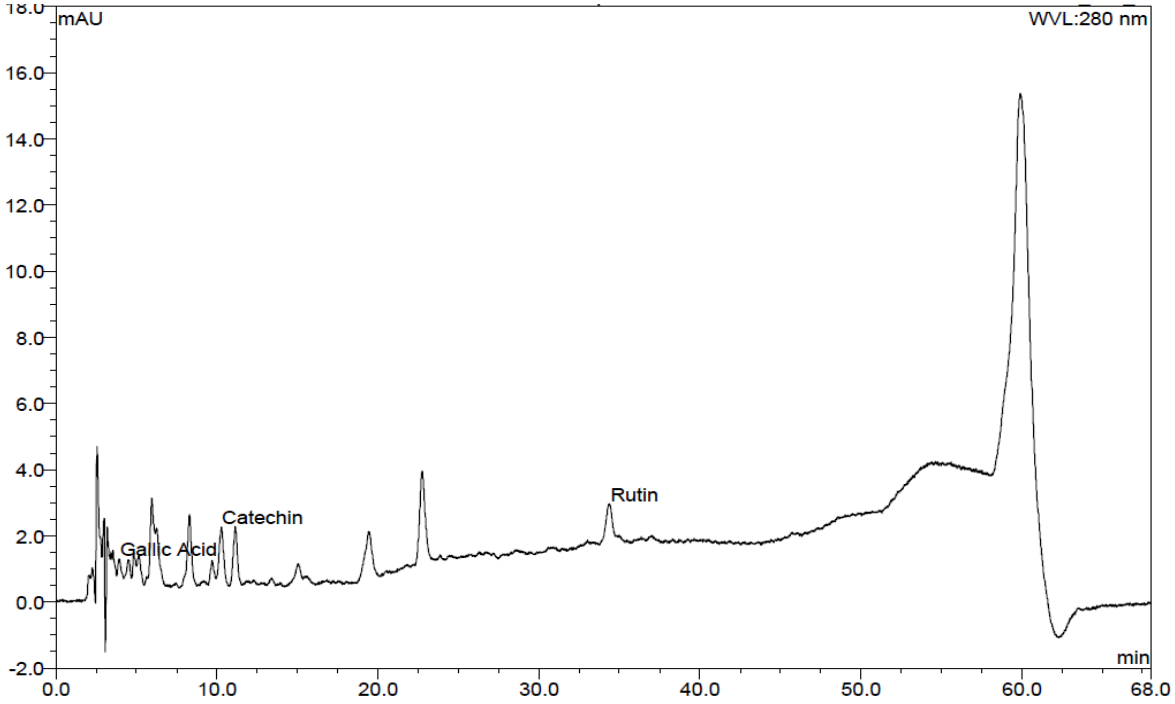


a. Control (No pectinase)

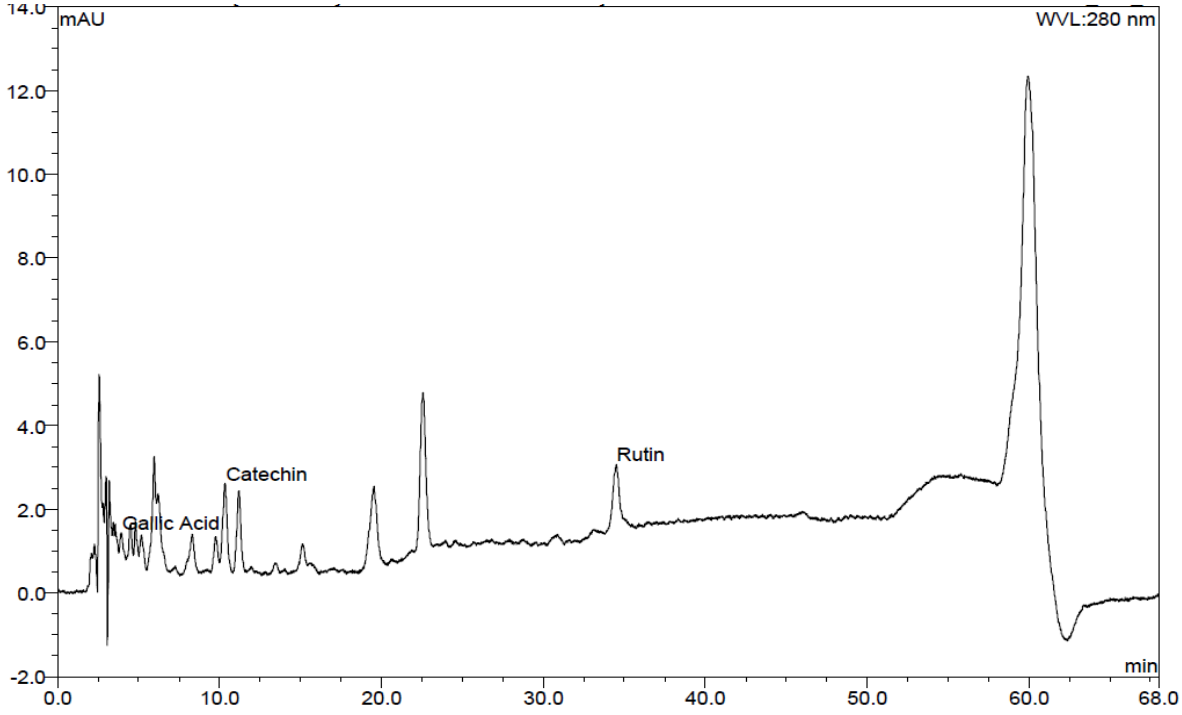


b. Pectinase treatement

Fig 5.10: Frontenac pomace HPLC chromatograms for the detection of Gallic acid, Catechin and Epicatechin at λ=280 nm for the pectinase treatment

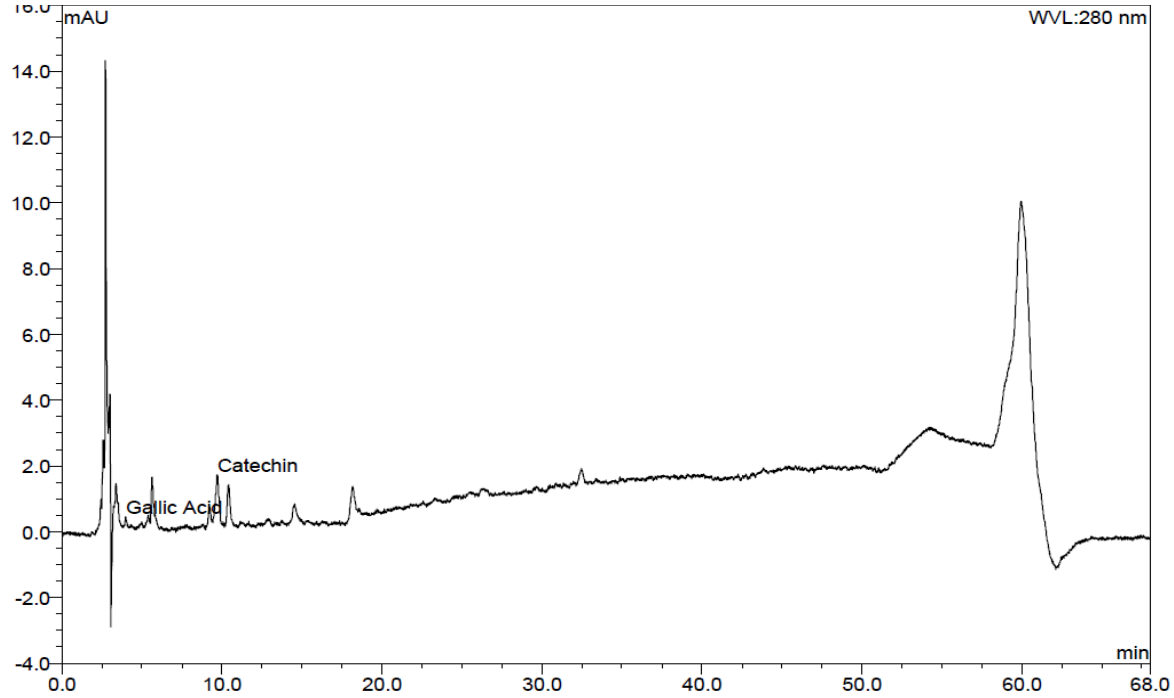


a. Control

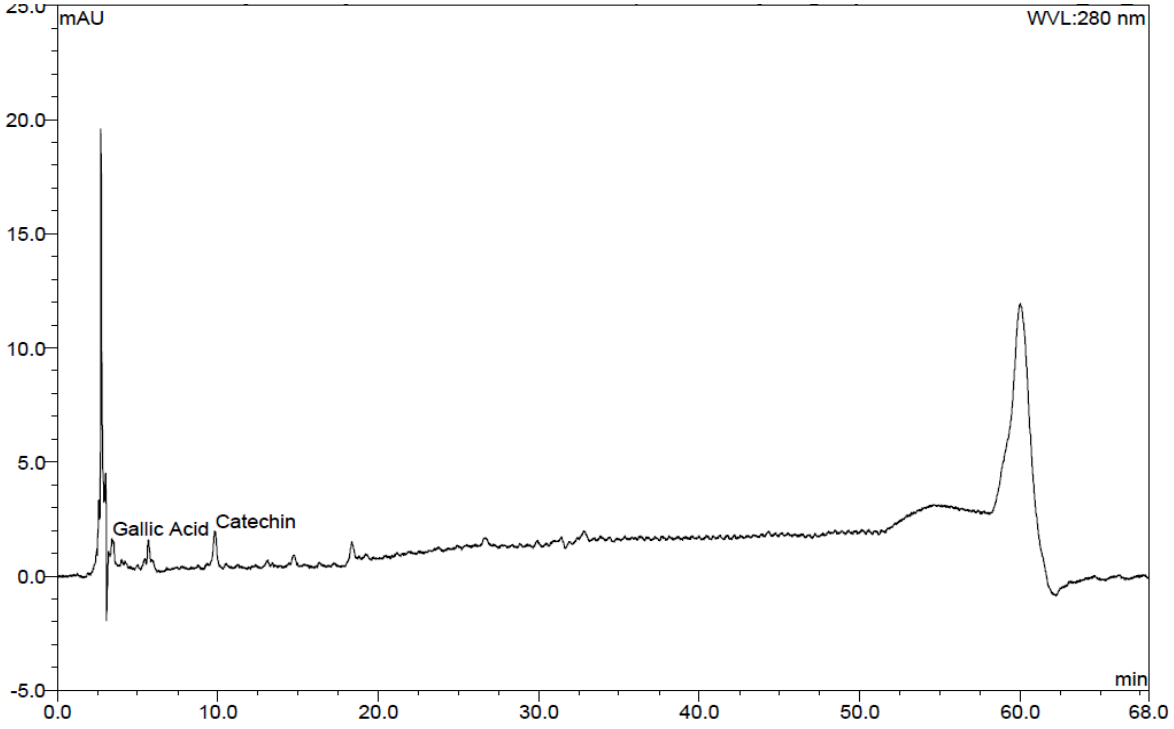


b. PEF treatment

Fig 5.11: St. Pepin pomace HPLC chromatograms for the detection of Gallic acid, Catechin and Epicatechin at λ=280 nm for the PEF treatment

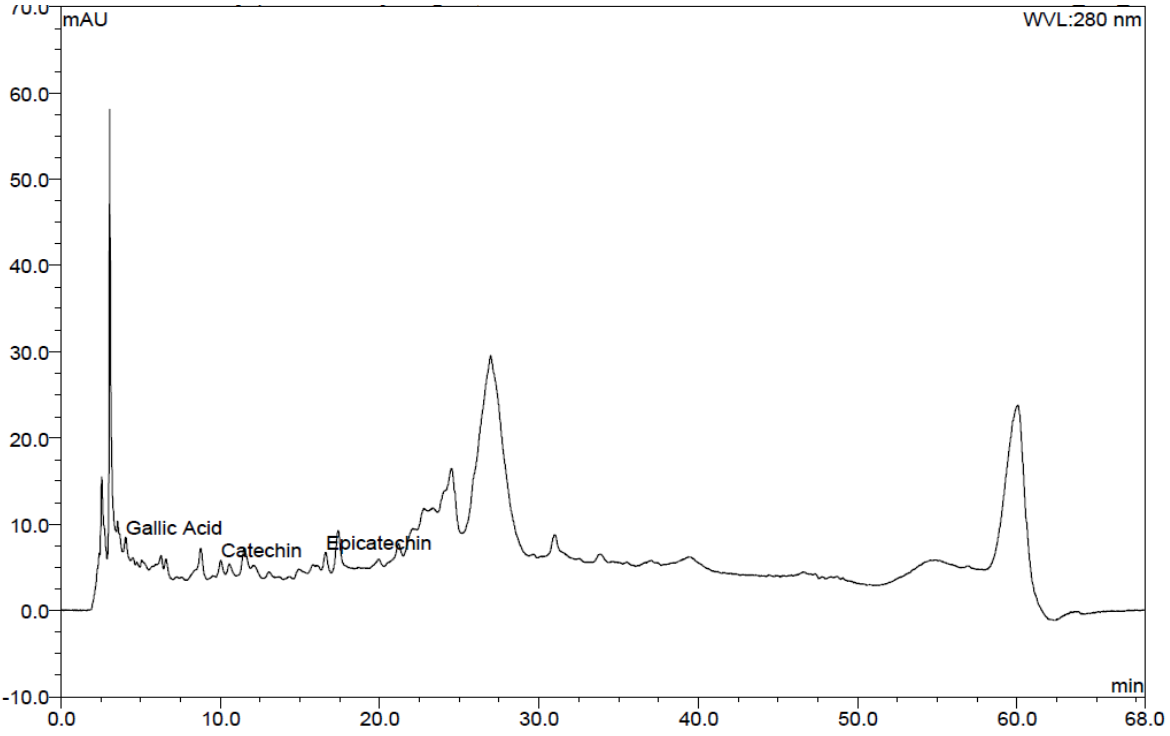


a. Control

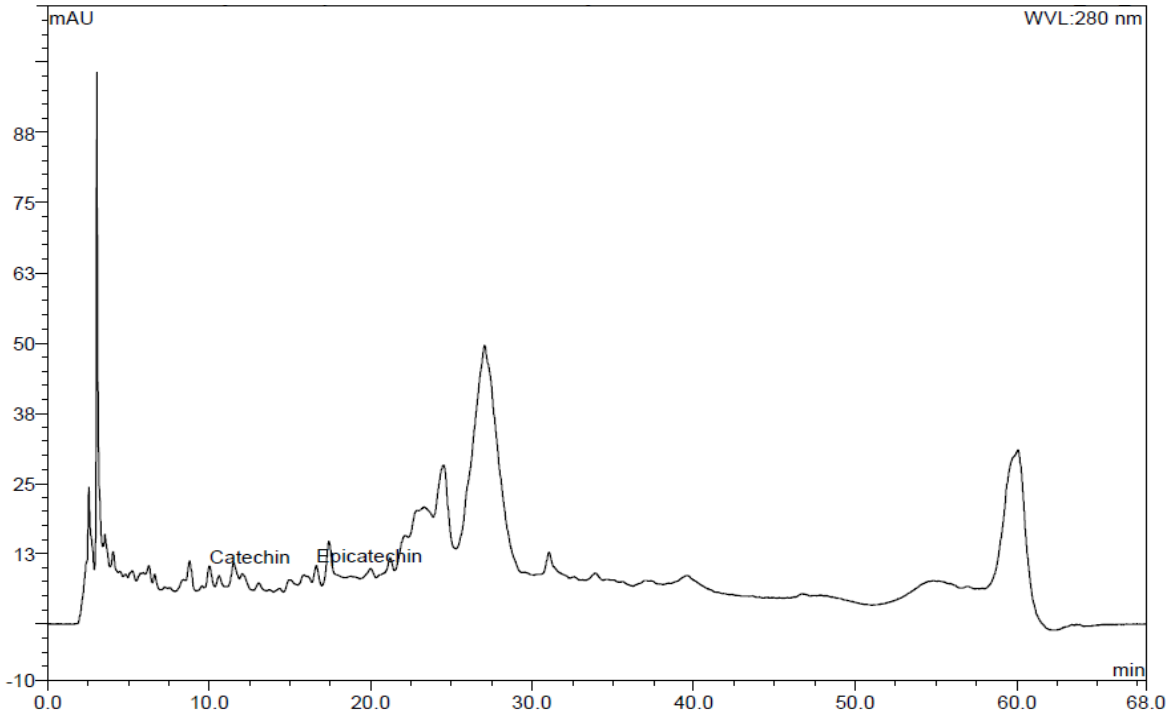


b. Pectinase treatment

Fig 5.12: St. Pepin pomace HPLC chromatograms for the detection of Gallic acid, Catechin and Epicatechin at λ=280 nm for the pectinase treatment

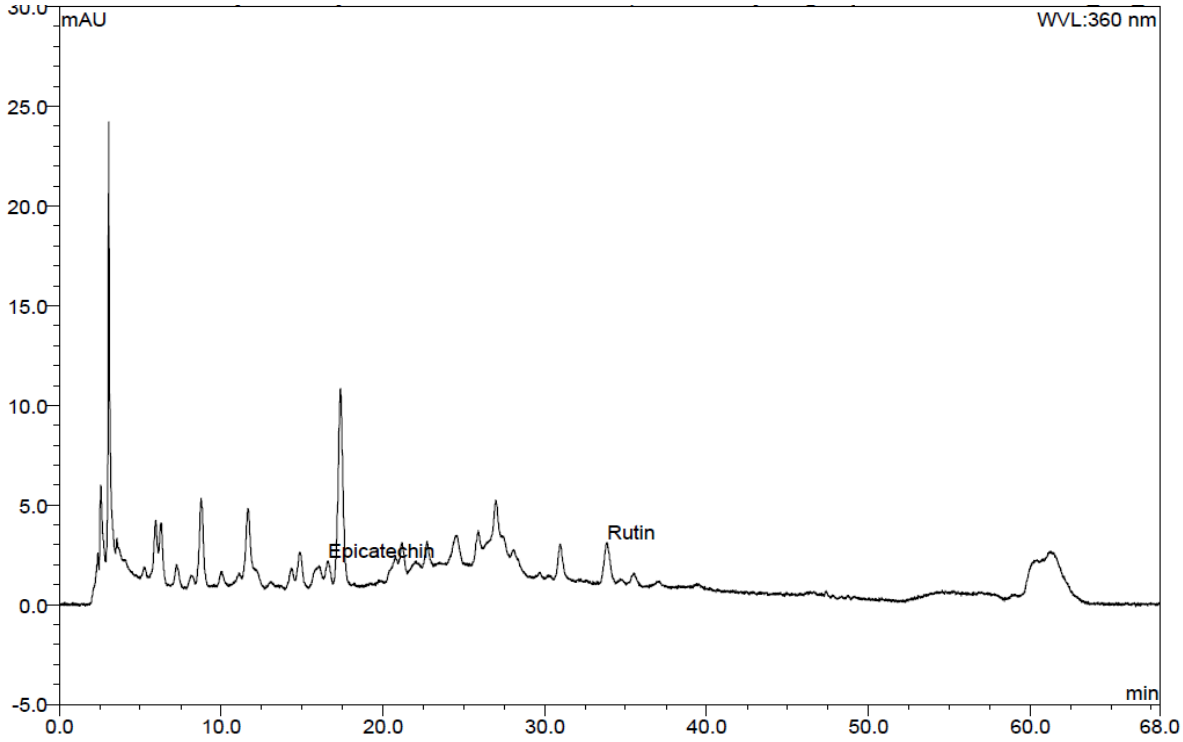


a. Control (No PEF)

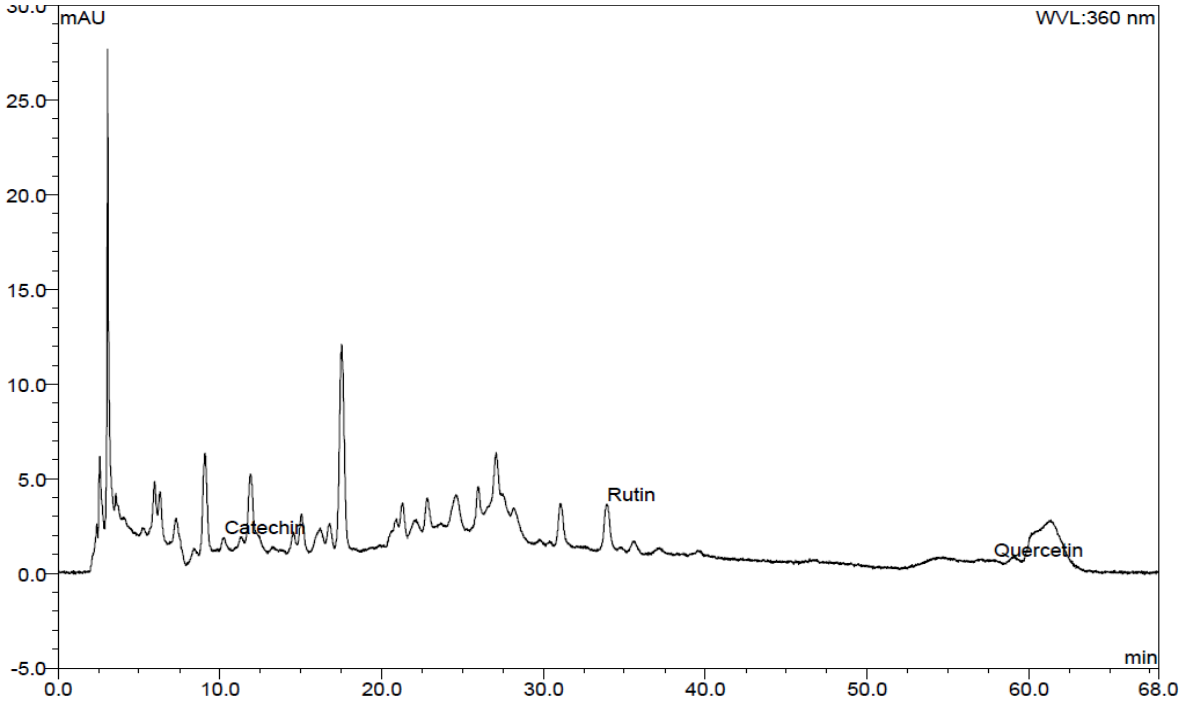


b. PEF treatment

Fig 5.13: St. Croix pomace HPLC chromatograms for the detection of Gallic acid, Catechin and Epicatechin at λ=280 nm for the PEF treatment

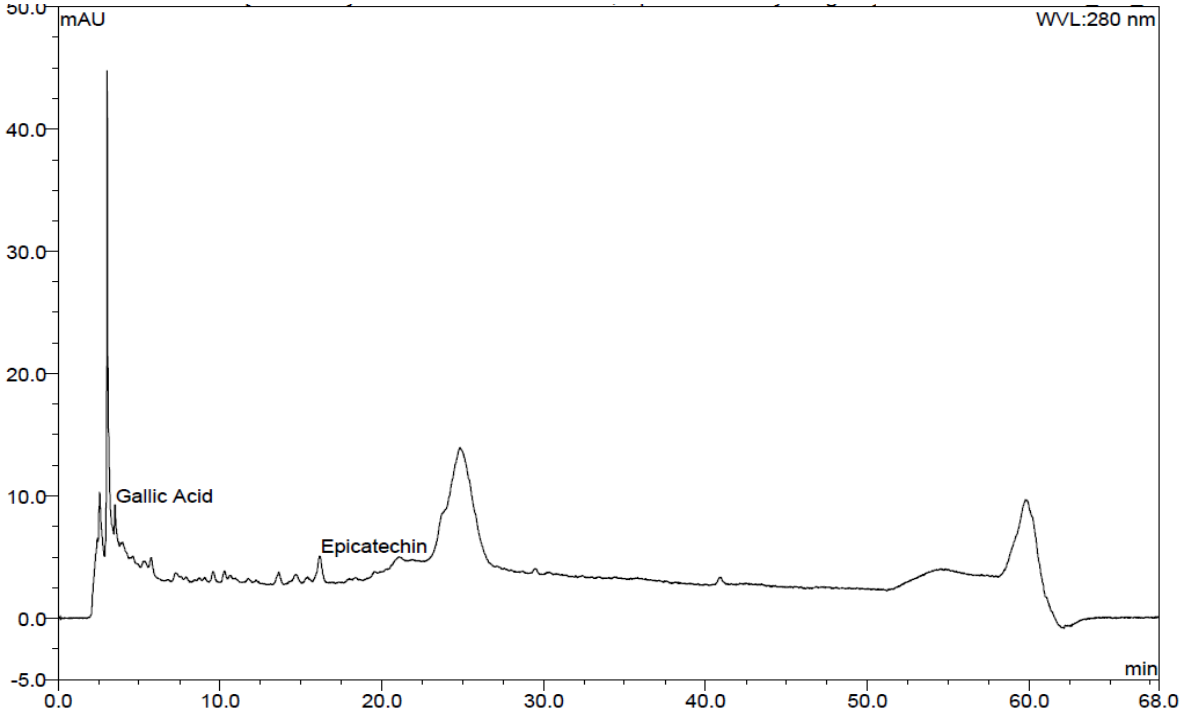


a. Control

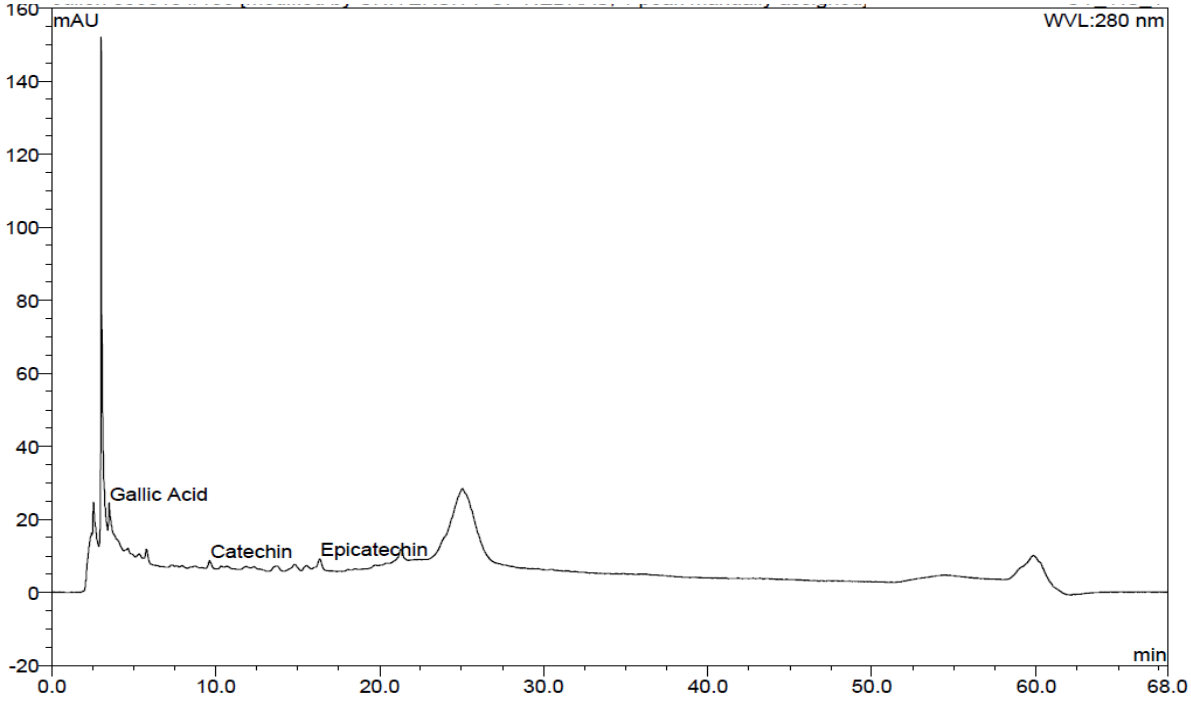


b. PEF treatment

Fig 5.14: St. Croix pomace HPLC chromatograms for the detection of Rutin, Quercetin and Myricetin at λ=360 nm for the PEF treatment

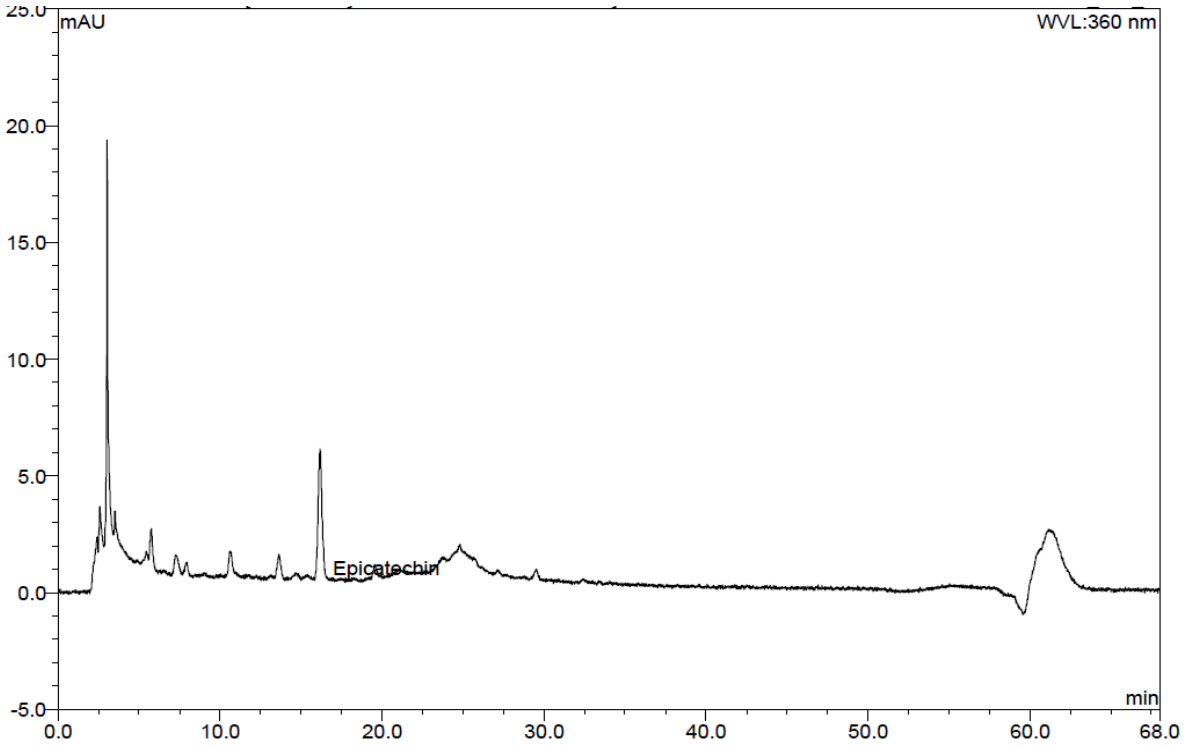


a. Control (No pectinase)

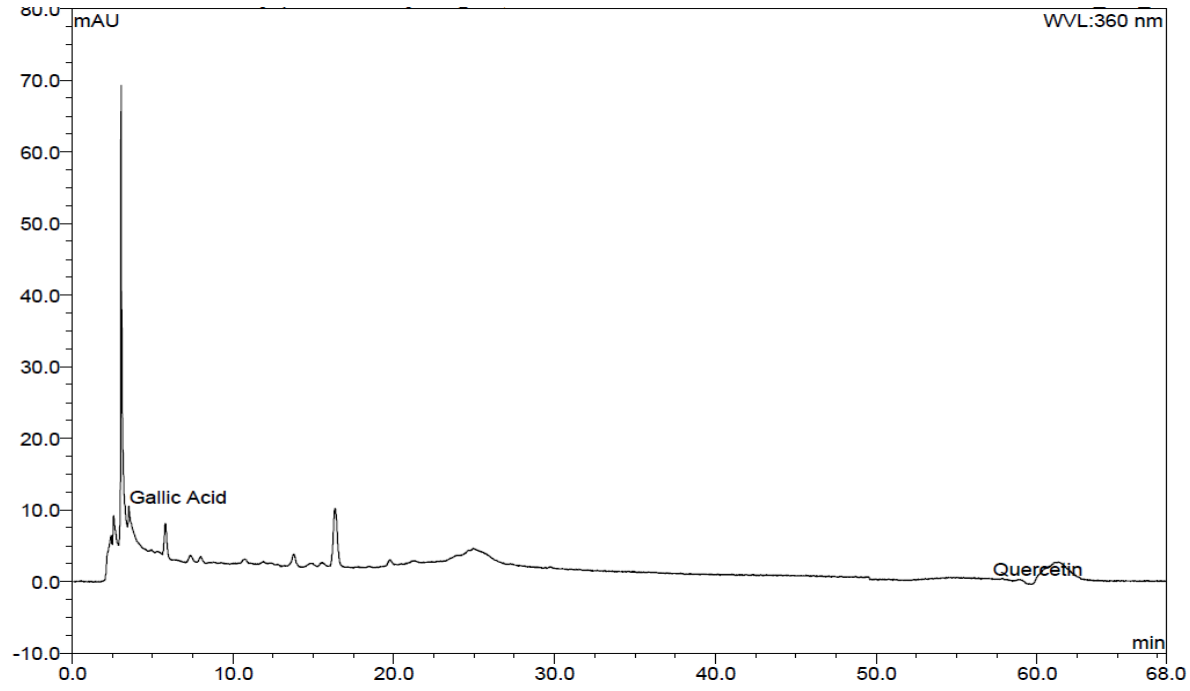


b. Pectinase treatment

Fig 5.15: St. Croix pomace HPLC chromatograms for the detection of Gallic acid, Catechin and Epicatechin at λ=280 nm for the pectinase treatment



a. Control



b. Pectinase treatment

Fig 5.16: St. Croix pomace HPLC chromatograms for the detection of Rutin, Quercetin and Myricetin at λ=360 nm for the pectinase treatment